STANDARDIZATION AND ANTIMICROBIAL ACTIVITIES ON SOME INDIAN MEDICINAL PLANTS

A THESIS SUBMITTED TO BUNDELKHAND UNIVERSITY
FOR THE AWARD OF THE DEGREE OF

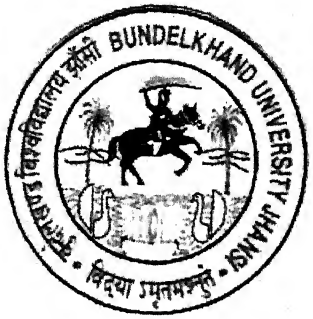
DOCTOR OF PHILOSOPHY

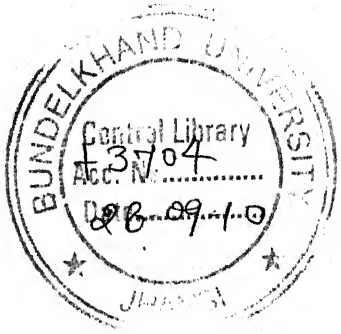
IN

PHARMACOGNOSY AND PHYTOCHEMISTRY

BY

SHYAM KRISHAN GUPTA
M.Pharm.





INSTITUTE OF PHARMACY
BUNDELKHAND UNIVERSITY
JHANSI (U.P.)
2006

INSTITUTE OF PHARMACY
BUNDELKHAND UNIVERSITY,
JHANSI

CERTIFICATE

This is to certify that the thesis entitled "**STANDARDIZATION AND ANTIMICROBIAL ACTIVITIES ON SOME INDIAN MEDICINAL PLANTS**" submitted to the **Bundelkhand University, Jhansi (U.P.)**, in fulfillment of requirements for the award of degree of **Doctor of Philosophy in Pharmacognosy and Phytochemistry**, embodies the original research work carried out by **Mr. Shyam Krishan Gupta** under our supervision. This work has not been submitted in part or full for the award of any other degree of this or any other university. That the Candidate has put in an attendance of more than 200 days with me.

3/8/06

Prof. P. K. Sharma
Principal
KIET School of Pharmacy
Ghaziabad
Ex- Director and Head
Institute of Pharmacy
Bundelkhand University
Jhansi (U.P.)

Supervisor

3/8/06

Prof S. H. Ansari
Dept. of Pharmacognosy
and Phytochemistry
Faculty of Pharmacy
Jamia Hamdard
New Delhi

Co-Supervisor

DECLARATION

I hereby declare that the thesis entitled **"Standardization and Antimicrobial Activities on some Indian Medicinal Plants"** embodies the results of the original research work carried out by me in the Institute of Pharmacy, Bundelkhand University, Jhansi and Dr. K.N. Modi Institute of Pharmaceutical Education and Research, Modinagar. This work has not been submitted in part or full for the award of any other degree of this or any other university.

Shyam Krishan Gupta
Reader
Dr. K.N. Modi Institute of Pharmaceutical Education and Research,
Modinagar- 201201

ACKNOWLEDGEMENTS

I consider this as an auspicious occasion to express my sincere gratitude to all the people who have helped me directly and indirectly in completion of this research work.

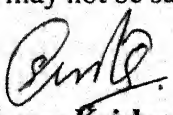
I wish to express my deep sense of gratitude to my Research Supervisor, **Prof. P. K. Sharma, Ex-Director and Head, Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.),** for his sagacious guidance, encouragement and providing the necessary facilities to carry out present study. His scholarly suggestions, immense interest, unstinting help and affectionate behaviour have been a fountain of inspiration to me.

Words fail me to thank my Co-Supervisor, **Prof. S.H. Ansari, Dept. of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi** for his liberal and incessant help and for the meticulous guidance and encouragement he rendered to me throughout my research work. I have been fortunate to be inspired by his affectionate behaviour and dynamic innovative nature throughout the period of my study.

It is great privilege to express my thanks to the teaching and non-teaching staff of Bundelkhand University, Jhansi for direct or indirect help.

I also wish to express my gratefulness to the staff of **Central Instrumentation Facilities, Faculty of Pharmacy, Jamia Hamdard, New Delhi,** for providing the HPTLC facilities.

I will be selfish if I will forget to put my heartiest thanks to my wife **Mrs. Saroj Gupta** without whose kind, untiring help and support I may not be successful in achieving this goal.



Shyam Krishan Gupta

CONTENTS

CHAPTER- 1

INTRODUCTION

STANDARDIZATION AND ANTIMICROBIAL ACTIVITIES ON SOME INDIAN MEDICINAL PLANTS

INTRODUCTION

India has rich flora of medicinal plants and these medicinal plants have been used in our traditional system of medicine, having very potent therapeutic activity but some of the medicinal plants used in our traditional system have not been fully investigated for therapeutic activity. Following three plants have been selected for the standardization and antimicrobial activities.

S.No.	Botanical Name	Common Name	Plant part to be used for studies
1.	*Tribulus terrestris* Linn.	Chotagokhru	Fruit
2.	*Cichorium intybus* Linn.	Kasni	Seed
3.	*Dolichos biflorus* Linn.	Kulthi	Seed

1. *TRIBULUS TERRESTRIS* LINN.

Tribulus is a genus of ascending or prostrate herb, belonging to the family Zygophyllaceae , distributed in the tropics and warm-temperate regions of the world. Three species which are found in India are *Tribulus terrestris, Tribulus cistoides* and *Tribulus alatus*. Among them *Tribulus terrestris* Linn. is an annual, upto 90 cm in length, commonly found throughout India upto, 5,400 m altitude[1].

The plant is commonly known in *Hindi:* Chotagokhru ; *Punjabi:* Bakhra ; *English :* Small caltrops.

It is a procumbent, ascending or suberect herb; stems and branches pilose, young parts silky-villous. Leaves opposite, abruptly pinnate, one of each pair usually smaller than the other , sometimes wanting altogether ; stipules lanceolate , hairy ; leaflets 3-6 pairs , oblong , mucronate , villous on both the surfaces ; base rounded oblique ; petioles minute , hairy. Flowers axillary or leaf-opposed, yellow, solitary, hairy; pedicles filliform. Sepals lanceolate, acute, hairy. Petals oblongobloid , claw short , hairy ; stamens 10 , inserted on the base of the disk , alternately longer and shorter , the latter with a small gland outside , filaments filliform , naked ovary sessile , hirsute , 5-12 lobed and celled ; style short ; stigmas 5-12 ; ovules superposed. Fruit globose with 5-hairy woodycocci, each with 2 spines. Seeds many in each coccus, with transverse partitions between them. Flowering and fruiting-hot season and rainy season (Fig.. 1).

Leaves are diuretic, tonic; increase the menstrual flow; cure gonorrhoea; a decoction is useful as a gargle for mouth trouble and painful gum and reduce inflammation.

The fruit is diuretic removes gravel from the urine and stone in the bladder. They are regarded as cooling, diuretic, tonic and aphrodisiac, and are used in painful micturition, calculous affections, urinary disorders and impotence. In some countries they are reputed tonic and astringent, used for coughs, scabies, anaemia and opthalmia.

The root is good stomachic and appetiser , diuretic and carminative.

The entire plant, but more particularly the fruits are used in medicines. It was given a good trial in Bright's disease with dropsy. The diuretic property of the drug is due to the presence of large quantities of nitrates present as well as the essential oil which occurs in the seeds[2].

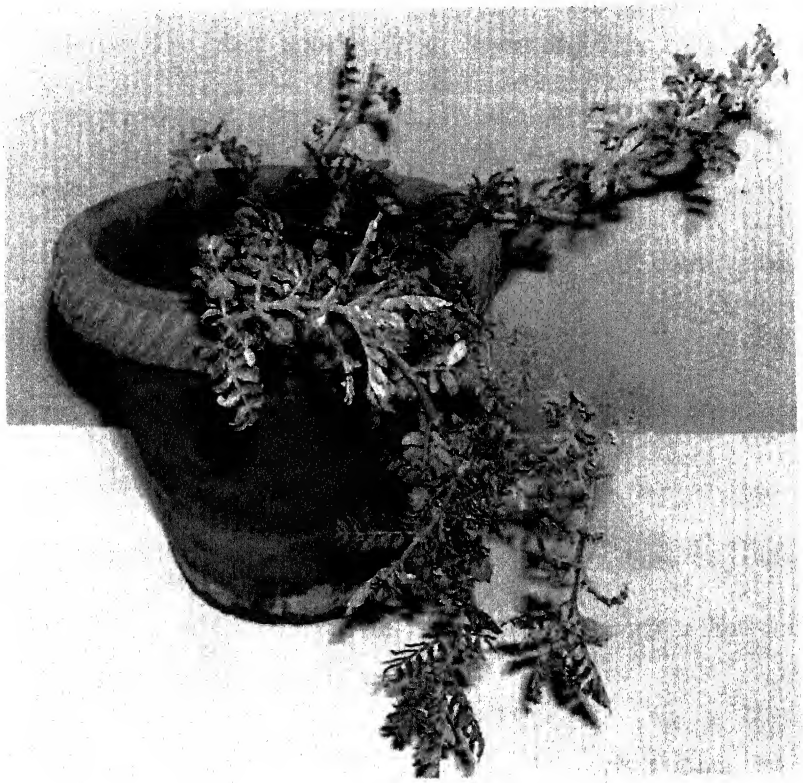


Fig.. 1: *Tribulus terrestris* grown in a pot

2. *CICHORIUM INTYBUS* LINN.

Cichorium is a genus of thirteen species belonging to the family compositae. Two species , viz., *C. endivia* and *C. intybus*, are of common occurance , cultivated throughout India , also grows wild in Punjab , north west India and Hyderabad in areas upto 6000 ft. elevation , waziristan , Baluchistan , W. Asia and Europe. It is grown either for fodder or as is more often the case, for the roots which form an article of commerce. The plant appears to grow on any type of soil. The dried root after roasting and powdering is used for mixing with coffee.

Cichorium intybus is an erect , glandular , perennial herb up to 90 cm in hight , with tough , rigid , spreading branches ; leaves radical and lower pinnatifid , lobes toothed , upper alternate , small , entire ; flowers bright blue in ligulate heads , terminal , axillary and clustered ; fruits smooth angled achenes , crowned with a ring of erect pappus scales (Fig.. 2).

The plant is commonly known in *Hindi :* Kasni ; *Punjabi :* Hand ; *Kannada :* Kacani ; *Malayalam :* Cikkari ; *Tamil :* Kasini ; *Sanskrit :* Kasani ; *English :* Chichory.

Cichorium intybus Linn. has been described to be of great medicinal value. There are two varieties of this species:

1. *Cultivated-sweet-variety:* The plant is a good tonic; cooling; useful in thirst, headache, ophthalmia, throat inflammation, enlargement of the spleen, fever, vomiting, diarrhoea. The root is the best part of the plant; good stomachic and diuretic; enriches and purifies the blood; lessens inflammation and pain in the joints. The leaves are applied topically to lessen pain in the joints. The seeds are

Fig.. 2: *Cichorium intybus* grown in a pot

tonic to the brain, alexiteric, appetiser; good in headache, ophthalmia, biliousness, lumbago, troubles of the spleen and asthma.

2. *Wild-bitter-variety :* The plant is tonic , emmenagogue, alexiteric; astringent to the bowels ; cures asthma , biliousness , inflammation ; enriches the blood . The root has tonic, demulcent and cooling properties. The seeds are considered carminative and cordial. A decoction is used in obstructed menstruation and for checking bilious vomiting. Flowers made into sherbet are given in liver disorders [3-5].

3. *DOLICHOS BIFLORUS* LINN.

Dolichos is a well known and wide spread genus of twining herbs of the family Leguminosae (Papillionaceae) occuring mainly in the tropical countries. It occurs all over India up to an altitude of 5000 ft. About 14 species occur in India, of which *D. biflorus* (Horse gram), *D. lablab* (Bean), *D. catijang* (Cow gram) , *D. Pruriens* (Cow hedge) and *D. soja* (Soya bean) are extensively cultivated and its seeds are used as food and leaves and stem as fodder.

Several varieties of horse gram differing in the colour of seed coat and the period of maturity are known under cultivation. The seeds are brown, light red, grey , black or mottled . The cultivated crop is usually a mixture of several varieties[6].

The plant is commonly known in *Hindi :* Kulthi ; *Sanskrit:* Kulastha; *Bengali:* Kulti, kurti kalai; *Marathi:* Kulith, Kulthi; *Gujarati:* Kulti; *Malayalam:* Kullu, kollu; *Telugu:* Wulavulu; *Tamil:* Kollu; *English:* Horse gram.

Dolichos biflorus is a branched sub-erect or trailing annual , with small trifoliate leaves , bearing , when mature , narrow , fat , curved pods , 1½ –2 in. long , tipped with a persistent style. The stems are very wide climbing slender, slightly pubsescent, oblong blunt, subglabrescent leaflets on a petiole, lateral ones very unequal sided, stipullae minute and linear. Flowers are 1–3 on very short pedicels in

the axils of the leaves. Calyx slightly downy with upper teeth quite connate, the side lanceolate and the lowest one linear. Corolla yellow. Pods are linear, subsessile, nearly straight, glabrous, 6 - 8 seeded, tipped with a persistant style (Fig.. 3).

The seeds of *Dolichos biflorus* have been used in the indigenous system of medicine for a long time as astringent to the bowels , fattening , antipyretic , anthelmintic , nerve tonic , diuretic , appetizing , aphrodisiac , emmenagogue etc. and cures "Kapha" and "Vata" , tumours , asthma, bronchitis , urinary discharges, hiccough , abdominal complaints , heart troubles , diseases of the brain , eye diseases , piles , leucoderma , liver troubles , leucorrhoea , menstrual derangements and removes stones from the kidney[7-10].

These medicinal plants are very much used in traditional system of medicine and many pharmacological properties have been attributed to various parts of these plants. The standardization of these plants parts are essential in order to prevent adulteration and admixture in the preparation of Ayurvedic medicine.

Hence the useful parts of these medicinal plants will be subjected for standardization and the extracts isolated from these plants parts will be screened for antimicrobial activities.

Fig.3: *Dolichos biflorus* grown in a pot

CHAPTER- 2

LITERATURE REVIEW

LITERATURE REVIEW

(i) *TRIBULUS TERRESTRIS* LINN.

Phytochemical Investigations

Fruit contains an alkaloid in traces (0.001%); fixed oil 3.5% consisting mainly of unsaturated acids, essential oil in very small quantities resins and fair amounts of nitrates[2].

Harman occurs in the herb and harmine in seeds. The plant contains saponins which on hydrolysis yield steroidal sapogenins. Kaempferol, keempferol-3- glucoside, kaempferol-3-rutinoside and a flavonoid tribuloside have been isolated from leaves and fruits[11].

Nath *et al.* reported crude protein 12.06%; ether extract 2.61%; crude fibre 27.7%; nitrogen free extract 40.83% ; total carbohydrates 68.61% ; total ash 16.72% ; calcium 4.21% and phosphorus 0.24%[12].

Earlier investigations of this plant yielded a number of steroidal sapogenin viz., chlorgenin, gitogenin, diosgenin and ruscogenin. Tomowa *et al.* identified a new sapogenin viz., tigogenin[13].

Purushothaman, *et al.* isolated two new steroid sapogenins, hecogenin and neotigogenin[14].

Mahato, *et al.* found β-sitosterol, stigmasterol and neotigogenin in whole plant of *T. terrestris* L[15].

Altogether 22 amino acids viz., Glutamic acid, Glutamine, Aspartic acid, Asparagine, Cystine, Cysteine, Tryptophan, Serine, Proline, Glycine, Alanine, Valine, Methionine, Leucine, Isoleucine, Tyrosine, Phenyl alanine, γ-Aminobutyric acid,

Ornithine, Lysine, Histidine and Arginine were identified in the root nodules of *T. terrestris* L. by Ather, *et al.*[16].

Duhan *et al.* reported a rich source of calcium in the leaves of *T. terrestris* L[17].

Afria showed that young leaves possessed the maximum concentration of protein (92.5 mg/g. dry wt.) and most of the individual free amino acids as compared with mature leaves and immature fruits[18].

Saleh *et al.* detected 25 flavonoid glycosides which belong to the common flavonols, kaempferol , quercetin and isorhamnetin with the 3-gentiobiosides as the major glycosides in *T. terrestris* L[19].

Singh *et al.* isolated Diosgenin and Tigogenin from over ground part of *T. terrestris* L[20].

Prakash, *et al.* confirmed 4 beta-carboline alkaloids, harmine , harmaline , harmane and tetrahydroharmine in the plant *T. terrestris*.[21]

Bourke, *et al.* identified beta-carboline alkaloid harmane and norharmane in the aerial parts of *T. terrestris*[22].

Zafar, R. *et al.* isolated diosgenin , hecogenin , ruscogenin and spirosta-3,5-diene from flowers of *T. terrestris* L[23].

Two compounds of cinnamic amide derivative named terrestriamide and 7-methyl hydroindanone-1, were isolated from *T. terrestris* L[24].

Pharmacological Activity

The plant *T. terrestris* L. is one of the most important ingredients of an Ayurvedic preparation. The drug is diuretic, tonic, aphrodisiac, blood purifier and often used to painful micturition, to remove 'tridosh', to cure skin and heart disease. The freshly expressed juice of the aqueous extract of the whole plant contains

inorganic nitrites, mostly potassium nitrite in toxic amounts. It is also used for the treatment of piles, cough, calculi and leprosy.

Chakraborty, *et al.* studied the various pharmacological action and reported that an alcoholic extract of the plant produced a sharp vasodepression in an anaesthetised dogs mediated through cholinergic mechanism. It also possessed some characteristics changes in C.N.S. and in Carbohydrate metabolism[25].

Prakash, *et al.* reported marked C.N.S. stimulant activity in adult albino mice in *T. terrestris* L[26].

Bourke, *et al.* observed locomotor disorders in sheep with the *T. terrestris* L. due to beta-carboline alkaloid[27].

Bourke *et al.* administered harmane and norharmane from alkaloidal extract of *T. terrestris* L. to normal sheep and showed that both compounds were able to cause locomotor effects in sheep[28].

Anand *et al.* found antiurolithiatic activity in albino rats in alcoholic extract of *T. terrestris* L[29].

Singh, *et al.* evaluated the diuretic action with minimal side effects on albino rat in *T. terrestris* L[30].

Administration of the fractions of ethanolic extract of the fruits of *T. terrestris* resulted in a varying degree of reduction in deposition of stone in albino rats[31].

Sangeeta *et al.* observed the effect of an aqueous extract of *T. terrestris* on the metabolism of oxalate in male rats fed sodium glycolate that lowering hyperoxaluria seemed to be mainly mediated through its inhibitory action on GAO and GAD, and its enhanced production of glyoxylate[32].

Vijaya , *et al.* examined in-vitro that aqueous extract of *T. terrestris* L. inhibited amylase and activated lipase digestive enzyme[33].

Antimicrobial Activity

Singh, *et al.* reported antibacterial activity against *E.Coli* in alcoholic extract of fruit of *Tribulus terrestris*[34].

Ikram , *et al.* reported , negligible activity in stem and leaf extracts of *T. terrestris* against *Escherichia Coli* , *Bacillus subtilis* , *Shigella dysenteriae* and *Salmonella typhi* as compared to streptomycin[35].

Antimicrobial activity was reported in an ethyl ether and 50% ethanolic extracts of *Tribulus terrestris* shoot against *Staphylococcus aureus* [36].

(ii) *CICHORIUM INTYBUS* LINN.

Phytochemical Investigations

Seeds contain a bland oil, 4.5% : fresh roots contain moisture, 77% gummy matter, 7.5%; glucose, 1.1%; bitter extractive, 4.0%; fat, 0.6%; cellulose, inulin and fibre, 9.0% and ash, 0.8%. The ash of the roots and also of the leaves is rich in potash. Betaine and choline are also present in small concentrations. Flowers contain a colorless crystalline glucoside; cichoriin, bitter substances lactucin and intybin[37-39].

Barakat, *et al.* reported mean of ferric iron content 3.4 mg % and cupric copper content 0.17 mg % by iodometric method in *Cichorium intybus* L[40].

Balbaa, *et al.* reported the presence of flavonoids, catechol tannins, glycosides, carbohydrates, unsaturated sterols, triterpenoids and the absence of alkaloids, oxidase enzyme and saponins in the roots of each of the eight varieties of *C. intybus* L[41].

Wight, *et al.* determined reducing sugers, sucrose and inulin content in roots of *C. intybus* L.[42].

The major anthocyanin of red leaves of *Cichorium intybus* has been identified as cyanidin 3 - 0 - β - (6-0-malonyl)-D-glucopyranoside by fast atom bombardment mass spectrometry and NMR spectroscopy [43].

Takeda, *et al.* identified a pigment, Delphinidin 3-(6-malonyl glucoside)-5-malonylglucoside in blue flowers of *C. intybus* L[44].

Cichorium intybus L. seed oil (5.8%) was examined for its physico-chemical values and fatty acid composition by gas chromatography. The oil was fractionated by TLC into lipid classes; neutral lipids (56.74%) and polar lipids (43.26%). Fractionation of neutral lipids gave hydrocarbon wax-esters (6.46%) , triglycerides (23.39%), free fatty acids (10.70%), 1, 3 - diglycerides (4.95%), 1, 2 - diglycerides (5.90%), 1 - monoglycerides (3.21%) and 2 - monoglycerides (2.13%). Polar lipids

were separated into glycolipids (30.22%) and phospholipids (13.04%). All the lipid classes except phospholipids were studied for their fatty acid composition. Except for 2 – monoglycerides, all other lipid classes showed a similar fatty acids pattern, as the saturated fatty acids constituted 72-88% of the total. All the lipid classes have shown a fair amount of an odd numbered fatty acid[45].

Grayer, *et al.* reported an antifungal phytoalexin , cichoralexin in leaves of *C. intybus* L[46].

Park, *et al.* isolated two known endesmanolides, magnolialide and artesin from the roots of *C. intybus* and their structures were identified as magnolialide (1β - hydroxyeudesma – 4, 13 – dien – 6, 12 - olide) and its 11β , 13 - dihydro derivative (artesin).[47]

The known eudesmanolide magnolialide and the known guainolide ixerisoside - D reported from *C. intybus,* along with the previously known sesquiterpene lactones, have also been isolated and identified by Kisiel , *et al.*[48].

Four anthocyanin pigments were isolated from flowers of *C. intybus* and identified as delphinidin 3 , 5 - di - 0 - (6 - 0 - malonyl - β - D - glucoside) and delphinidin 3 - 0 - (6 - 0 - malonyl - β - D - glucoside) - 5 - O - β - D - glucoside and the known compounds were delphinidin 3 - O - β - D - glucoside - 5 - 0 - (6 - O - malonyl - β - D - glucoside and delphinidin 3 , 5 - di - O - β - D - glucoside, in addition 3 - O - p - coumaroyl quinic acid has been identified by Norback , *et al.*[49].

Pharmacological Activity

Balbaa, *et al.* observed marked depression on the amplitude and on the rate of the isolated toad's heart in roots of each of eight varieties of *C. intybus* L. This type of effect was similar to quinidine.[41]

Pandey observed bradycardia in normal and hypodynamic heart of frog and a fall in B.P. with a corresponding increase in respiratory rates in dog treated with alcoholic extract of seeds of *C. intybus* L.[50].

Handa, *et al.* reported cholagoque activity in alcoholic extract of the *C. intybus* L.[51].

A significant decrease in the triglyceride level of liver, plasma and heart coupled with decreased cholesterol level in plasma was observed in rats, fed with high level of saturated fat(45%) supplemented with 5% roots of *C. intybus* L. as compared to high fat fed group, by Kaur *et al.*[52].

Misra, *et al.* found antimalarial activity against erythrocytic stages of *plasmodium berghei* only *in vitro* in alcoholic extract of seeds of *C. intybus* L.[53].

Gadgoli, *et al.* found hepatoprotective activity against carbon tetrachloride and paracetamol induced toxicity in rats , treated each with chloroform , methanol and water extract of seeds of *Cichorium intybus* L.[54].

Zafar *et al.* reported better antihepatotoxic effect against carbon tetrachloride induced hepatocellular damage in albino rats, treated with root callus extract as compared to the natural root extract of *Cichorium intybus* L.[55].

Antimicrobial Activity

Abou-Jawdah , *et al.* found antimycotic activity against phytopathogenic fungi in petroleum ether extract of *C. intybus* L.[56].

(iii) *DOLICHOS BIFLORUS* LINN.

Phytochemical Investigations

The seed has moisture , 11.8% ; crude protein 22.0% ; fat , 0.5% ; minerals , 3.1% ; fibre , 5.3% ; carbohydrates , 57.3% ; calcium , 0.28% ; phosphorus , 0.39% ; iron , 0.0076% ; nicotinic acid , 0.0015% ; carotene , 119 IU/100g. , arginine 6.0 - 7.1%, tyrosine 6.68% and lysine 7.64%. Other important constituents of *D. biflorus* are strepogenin, β-sitosterol, bulbiformin, linoleic acid (in the seeds oil, 30-60%), polyphenols, oxalates (40% soluble) and crude fibre (5.3%)[57-59].

Pant , *et al.* found moisture 10.58% ; ash 3.86% ; fat 2.26% and crude protein 21.35% in seeds[60].

Mahadevappa *et al.* reported palmitic acid , linoleic acid, oleic acid and linolenic acid in seed oil of *D. biflorus* L.[61].

An unusual enzyme allantoinase was isolated from germinated seeds of *D. biflorus* L. by Mary *et al.*[62].

Seeds of *D.biflorus* L. contain total lipids 1.7 - 2.2% , neutral lipids 46 - 52% of total lipids , glycolipids 10 - 12% and phospholipids 35 - 40% of total lipids. Its amino acid composition is aspartic acid , lysine , phenyl-alanine , glycine , threonine , alanine , tyrosine , valine , glutamic acid , leucine , proline , serine and tryptophan. Seeds are rich source of ribonuclease. The glycosidases β - H -acetylgluco - samanidase, α- and β-galactosidases, α-mannosidase and β-glucosidase have been isolated and purified. Haemagglutinin was isolated from the seeds by fractionation and characterized as a glycoprotein of molecular weight about 130000 with amino acids and carbohydrates (0.5% galactose , 0.2% mannose , rhamnose and fructose)[63].

A number of isoflavones have been isolated from the leaves and stems of *D. biflorus* L. namely Genistein , 2'-hydroxy genistein , dalbergioidin , kievitone ,

phaseollidin and isoferrerin after inoculation by some nonpathogenic bacteria , along with coumestrol and psoralidin[64].

Ingham , *et al.* isolated two minor isoflavonoids dolichin A and B from the bacteria inoculated leaves of *D. biflorus* L.[65].

Mitra , *et al.* isolated 5-Hydroxy-7,3',4'-trimethoxy - 8 – methyliso-flavone 5-neohesperidoside isoflavone from the ethanolic extract of seeds of *D. biflorus* L. [66].

Akihisa, *et al.* isolated and identified fourteen triterpene alcohols and one 3-oxosteroid , stigmasterone [(24R)-stigmast - 4 - en - 3 - one] and others were unidentified from seeds of *D. biflorus* L. [67].

Dubey *et al.* identified D – glucose , D – galactose , L - rhamnose , D - arabinose and L - ascorbic acid along with amino acids viz., glycine , alanine , serine , cystine and aspartic acid from seeds of *D. biflorus* L.[68].

Pharmacological Activity

The seeds are diuretic ; emmenagogue ; increase appetite ; remove stone from kidney ; cure hiccough , eye troubles , piles , enlargement of the spleen , pain in the liver ; improve the complexion ; cause biliousness. The decoction is used in leucorrhoea and menstrual derangement [69].

Kamboj , *et al.* reported that no anti-implantation activity at a dose of 200 mg/kg on days 1-7 post-coitum in rats in petroleum ether , alcohol and aqueous extracts of seeds of *D. biflorus* L.[70].

Laskar , *et al.* found antihepatotoxic activity in seeds of *D. biflorus* L. against paracetamol intoxicated rats at a dose of 10 mg/kg[71].

Antimicrobial Activity

Basak , *et al.* found antibacterial activity against *Pseudomonas aeruginosa* , *Escherichia coli* , *Proteus vulgaris* and *Bacillus subtilis* in methanolic extract of seeds of *D. biflorus* L.[72].

Looking to the medicinal utility of these plants in the literature mentioned above and comparatively pharmacognostic studies on the parts of these plants are very few and fragmentary. As pharmacognostic screening of the plant parts is essential for identification of the commercial sample; the same has been undertaken to standardize for prevention of admixtures and adulterants in the preparation of Ayurvedic formulation.

CHAPTER- 3

EXPERIMENTAL

EXPERIMENTAL

(A) STANDARDIZATION OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

MATERIALS AND METHODS

The fruits of *Tribulus terrestris* were procured from local market of Modinagar, Ghaziabad and were identified by Dr. H.B. Naithani , Botanist and Scientist , Forest Tree Seed Laboratory , Silviculture Division , Forest Research Institute , Dehradun. The fruits were shade dried.

(A) PHYTOCHEMICAL STUDY

1. Quality Parameters:

Moderately coarse powders of fruits were prepared by crushing the fruits in electric grinder for the proximate analysis. The brief description of the I.P. methods[73] used for the determination of Foreign Organic matter, loss on drying , Ash values , Extractive values and other Quality Parameters on fruit of *T. terrestris* Linn. are given below and results are tabulated in Table 1.

1. **Foreign Organic Matter:**

About 300 g of the original sample was weighed and spread it out in a thin layer. The sample was inspected with the unaided eye and foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was determined from the weight of the sample taken.

2. **Loss on drying:**

Loss on drying is the loss in weight in % w/w resulting from water and volatile matter of any kind that can be driven off.

About 1 g of the accurately weighed amount of the powdered drug was taken in a weighing bottle and dried the sample in an oven at 105° till the weight was constant.

3. **Total ash:**

About 2 g accurately weighed powder of the drug was taken in a silica crucible and incinerated by gradually increasing the heat at a temperature not exceeding 450° until free from carbon. The crucible was allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

4. **Acid-insoluble ash:**

The total ash obtained in above experiment was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water, ignited in a tared silica crucible. The crucible was allowed to cool in a desicator and weighed. The percentage of acid - insoluble ash was calculated with reference to the air dried drug.

5. **Sulphated ash:**

About 1 g accurately weighed powder of the drug was taken in a tared silica crucible and ignited gently at first , until the powder drug was thoroughly charred , cooled , moistened the residue with 1 ml of sulphuric acid and again heated gently until the white fumes were no longer evolved , reignited at 800 ± 25° till the weight was constant.

6. **Water-soluble ash:**

The ash of the powdered drug was obtained as mentioned above and boiled for 5 min with 25 ml of water , insoluble matter was collected on an ashless filter paper , washed with hot water and ignited for 15 min at a temperature not exceeded 450° . The difference in weight between the insoluble matter and the weight of the ash

represents the water - soluble ash. The percentage of the water-soluble ash was calculated with reference to the air dried substance.

7. **Ethanol-soluble extractive:**

5 g of the powdered drug was macerated with 100 ml of ethanol in a closed flask for 24 h shaking the contents of the flask frequently during the first 6 h and allowing standing for 18 h then filtered rapidly. 25 ml of this filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105° and weighed. The percentage of ethanol - soluble extractive was calculated with reference to the air dried drug .

8. **Water-soluble extractive:**

The same procedure as directed for the determination of ethanol - soluble extractive was adopted using chloroform water instead of ethanol.

9. **Petroleum ether-soluble extractive:**

The same procedure as directed for the determination of ethanol - soluble extractive was adopted using Pet. ether (60-80°) instead of ethanol.

10. **Chloroform-soluble extractive:**

The same procedure as directed for the determination of ethanol - soluble extractive was adopted using chloroform instead of ethanol.

11. **Volatile oil Content:**

50 g of fruit powder was boiled with water in a round bottomed flask fitted with Clevenger apparatus. The volume of volatile oil which being lighter than water remains on the top of the distillate was measured.[73-74]

TABLE 1: QUALITY PARAMETERS OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

S.No.	Quality Parameters	Value
1.	Foreign organic matter	1.662%
2.	Loss on drying	10.10%
3.	Total ash	12.79%
4.	Acid-insoluble ash	0.97%
5.	Sulphated ash	2.07%
6.	Water-soluble ash	5.79%
7.	Ethanol-soluble extractive	1.862%
8.	Water-soluble extractive	16.8%
9.	Petroleum ether-soluble extractive	1.018%
10.	Chloroform-soluble extractive	1.26%
11.	Volatile oil	Nil

2. Fluorescent analysis:

Very faint fluorescence in the alcoholic extract at short (254 nm) and long (366 nm) ultra-violet wavelengths was observed as shown in Table 2.

TABLE 2: FLUORESCENT ANALYSIS OF ALCOHOLIC EXTRACT OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

S.No.	Light Source	Wave length (nm)	Color observed
1.	Ultra – violet light	254	Faint
		366	-do-
2.	Ordinary light	-	Colorless

3. Behaviour of powdered drug with different reagents:

The behaviour of the fruits Power of *T.terrestris* with different chemical reagents was observed as shown in Table3.

TABLE 3: BEHAVIOUR OF THE FRUITS POWDER OF *TRIBULUS TERRESTRIS* LINN. WITH DIFFERENT REAGENTS

S. No.	Reagents	Observation
1.	Water	Colorless turbid soln.
2.	5% KOH	Brown colored turbid soln.
3.	Dil. HCL	Faint lemon yellow tinted soln.
4.	Dil. H_2SO_4	-Do-
5.	Dil. HNO_3	-Do-
6.	$Fecl_3$ soln.	Light brown precipitation
7.	Dragendorff's soln.	Orange brown precipitation
8	KI and I soln.	Light orange brown turbid soln.

4. Phytochemical Screening:

The fruits of *Tribulus terrestris* (130 g) were coarsely powdered and was successively extracted with petroleum ether (60 - 80°) , chroloform , ethanol and water in a Soxhlet extractor . The various extracts obtained were then subjected to qualitative tests for the presence of important plant constituents like alkaloids , carbohydrates , glycosides , phytosterols , saponins , tannins , proteins and amino acids , fixed oils and fats , gums and mucilages , and resins.

The tests applied are given below and the results obtained from the tests are tabulated in Table 4.

1. Alkaloids:

A small quantity of the extract was dissolved in Dil. Hydrochloric acid and the solution was filtered. The filtrate was tested with a number of alkaloidal reagents like Mayer's reagent, Dragendorff's reagent, Hager's reagent and Wagner's reagent.

2. Carbohydrates:

A small quantity of the extract was taken up in water and filtered. The filtrate was treated with Molisch's reagent , Fehling solution to detect the presence of carbohydrate.

3. Glycosides:

A little of the extract was hydrolysed with dilute hydrochloric acid and subjected to Legal's test and Borntrager's test for glycosides.

4. Phytosterols:

A little quantity of the extract was subjected to Liebermann's test , Libermann-Burchard's test to detect the presence of phytosterols.

5. Saponins:

A little quantity of the extract was subjected to Foam test to detect the presence of saponins.

6. Tannins:

A small quantity of the extract was boiled with distilled water and filtered. The filtrate was tested with ferric chloride and lead acetate soln. to indicate the presence of tannins.

7. Proteins and amino acids:

A small quantity of the extract was taken up in water and treated with Millon's reagent and Ninhydrin reagent to detect the presence of protein and free amino acids.

8. Fixed oils and fats:

A small quantity of the extract was subjected to spot test and saponification test to detect the presence of fixed oils and fats.

9. Gums and Mucilages:

A small quantity of the aqueous extract is slowly added to about 25 ml of 95% alcohol. A little brown precipitate indicates the presence of gums and mucilages.

10. Resins:

A small quantity of the extract was dissolved in 3 ml of acetone. 3 ml of hydrochloric acid was added to it and heated on a water bath for 30 minutes. Development of pink color, which on dilution gives magneta red color indicates the presence of resin[75].

TABLE 4: TESTS FOR COMMON PLANT CONSTITUENTS IN VARIOUS EXTRACTS OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

S.No.	Plant constituents / Test/Reagent used	Extracts			
		Petroleum ether (60-80°)	Chloroform	Alcohol	Water
1.	**Alkaloids**				
	a) Mayer's reagent	–	+	+	–
	b) Dragendoff's reagent	–	+	+	–
	c) Hager's reagent	–	+	+	–
	d) Wagner's reagent.	–	+	+	–
2.	**Carbohydrates**				
	a) Molisch's reagent	–	–	–	+
	b) Fehling's solution	–	–	–	+
3.	**Glycosides**				
	a) Legal's test	–	–	–	+
	b) Borntrager's test	–	–	–	–
4.	**Phytosterols**				
	a) Liebermann's test	–	–	+	–
	b) Liebermann-Burchard's test	–	–	+	–
5.	**Saponins**				
	Foam test	–	–	–	–
6.	**Tannins**				
	a) Ferric chloride solution	–	–	+	–
	b) Lead acetate solution	–	–	+	+
7.	**Proteins and amino acids**				
	a) Millon's reagent	–	–	+	+
	b) Ninhydrin reagent	–	–	+	+
8.	**Fixed oil and fats**				
	a) Spot test	+	–	–	–
	b) Saponification test	+	–	–	–

9.	**Gums and mucilages** Alcoholic precipitation	–	–	–	–
10.	**Resins**	–	–	+	–

5. Chromatographic analysis:

(A) Thin Layer Chromatography (TLC)

Preparation of extract:

5 g sample of powdered fruit was refluxed for 1 h with 50 ml Chloroform and filtered. The marc was refluxed for 1 h with 50 ml methanol and filtered. The filtrate was evaporated to dryness under vacuum. 50 ml of 2N hydrochloric acid was added to the residue and refluxed the solution in a heating mantle for 1 h. 1 g sodium bicarbonate was added after cooling the solution and extracted with three successive quantities of 20 ml of chloroform. Combined chloroform layers were washed with water, dried over anhydrous sodium sulphate and evaporated the solution to dryness under vacuum. The residue was dissolved in 2 ml of chloroform to be used as test solution.

Reference solution:

1 mg Diosgenin was dissolved in 4 ml methanol.

Solvent system:

Toluene: ethyl acetate (8:2)

The extract solution and reference solution were applied on silica gel G plate and visualized the spots in day light by spraying the plate with anisaldehyde-sulphuric acid reagent and heated at 120° for 10 min.[76-77].

A yellowish green spot having h*Rf*-value 29 corresponding to diosgenin was observed in both the extract and reference solution tracks. Other yellowish green spots

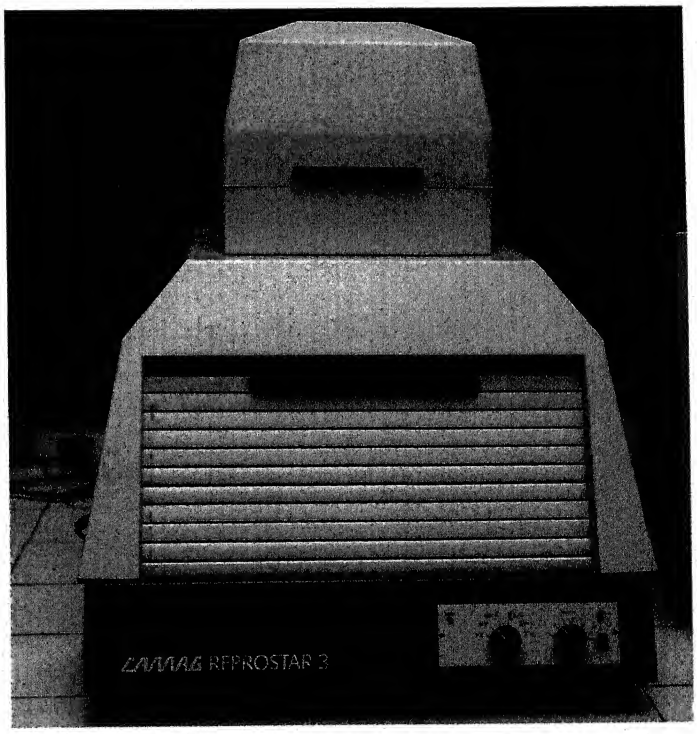
having h*Rf*-values 13 and 84 , prominent violet spots having h*Rf*- values 91, 53, 43, 34 and 21 and a dark blue spot having h*Rf*-value 14 were also observed in the extract solution as recorded in Table 5 .

TABLE 5: TLC OF CHLOROFORM EXTRACT OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

No. of Spots	**Color of Spots**	**Distance travelled by solvent (mm)**	**Distance travelled by solute (mm)**	***Rf* -values**	**h*Rf*-values**
1.	Violet	130	118.3	0.91	91
2.	Yellowish green	-do-	109.2	0.84	84
3.	Violet	-do-	68.9	0.53	53
4.	-do-	-do-	55.9	0.43	43
5.	-do-	-do-	44.2	0.34	34
6.	Yellowish green*	-do-	37.7	0.29	29
7.	Violet	-do-	27.3	0.21	21
8.	Blue	-do-	18.2	0.14	14
9.	Yellowish green	-do-	16.9	0.13	13

* The h*Rf*- value (29) of this spot resembles with that of diosgenin.

High performance thin layer chromatography



(B) High Performance Thin Layer Chromatography (HPTLC)

The air dried, pulverized fruits of *T. terrestris* were successively extracted with petroleum ether ($60\text{-}80^0$), benzene, chloroform, ethanol and water in a Soxhlet extractor. The extracts were subjected to HPTLC. Following steps were involved in HPTLC studies[78].

1. Application of sample:

Commercially available pre-coated silica gel $G60F_{254}$ TLC plate (10 X 10 cm, E. Merck, Germany) was used for the study. The different extracts were applied on plate in a single band width 6mm, using Camag Linomat 5, automatic sample applicator. 4µl sample of each extract was spotted on TLC plate under nitrogen stream by Camag Linomat syringe (100 µl). The plate was dried in air.

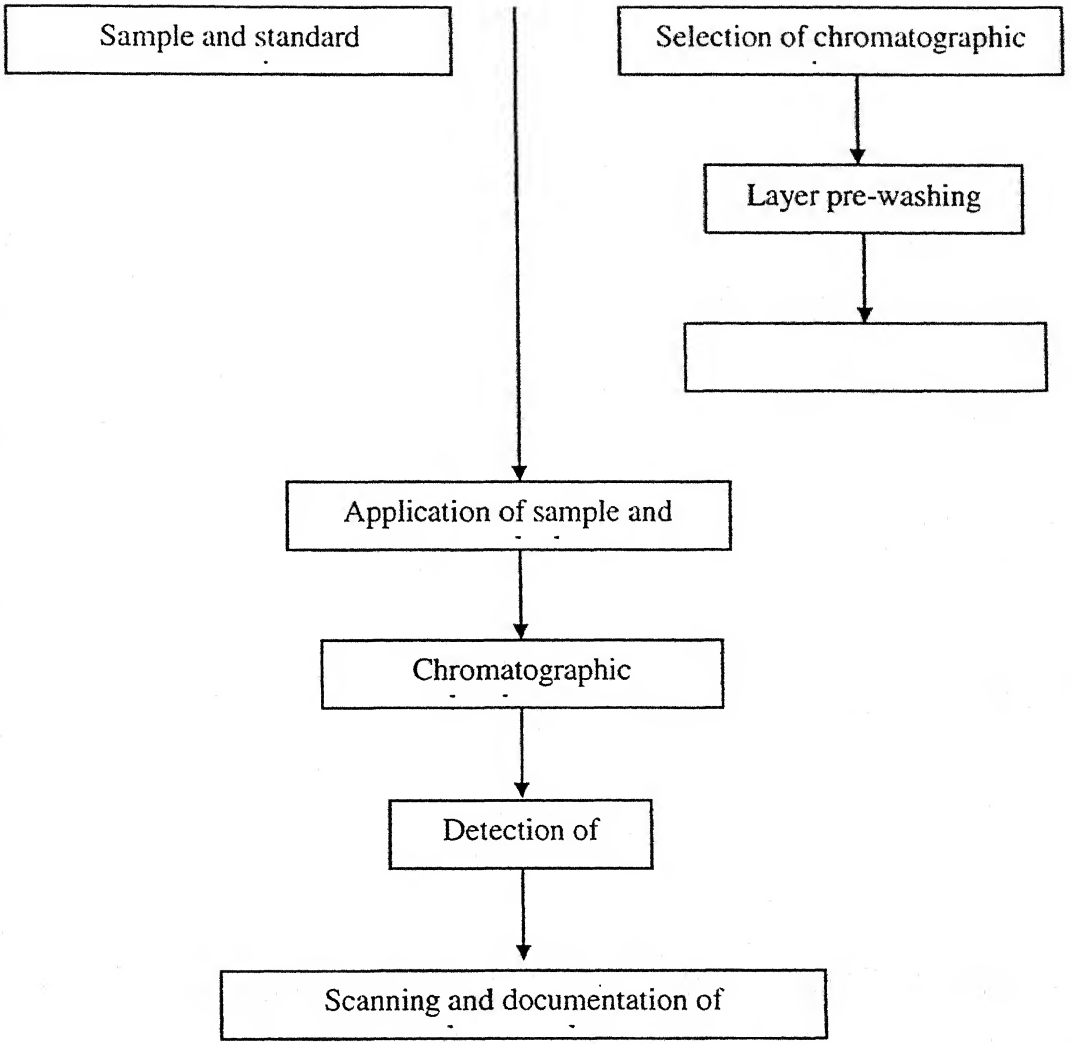
2. Chromatogram development:

The plate was developed up to 80 mm using mixture of Toluene: ethyl acetate (8:2) as mobile phase in a Twin-trough glass chamber (10X10 cm, Camag, Switzerland), previously saturated with mobile phase for 30 min. After developing, the plate was removed from the chamber, dried in air for 15 min and observed under UV Chamber, Camag Reprostar 3, at 366 nm as shows in Fig.. 4.

3. Densitometric scanning:

The developed plate was scanned using Densitometer, Camag TLC Scanner 3 at 366 nm.[78].

The HPTLC Chromatograms are shown in Fig.s. 5 to 9 and the number of components separated, their R_f values and percentage peak area are tabulated in Table 6.



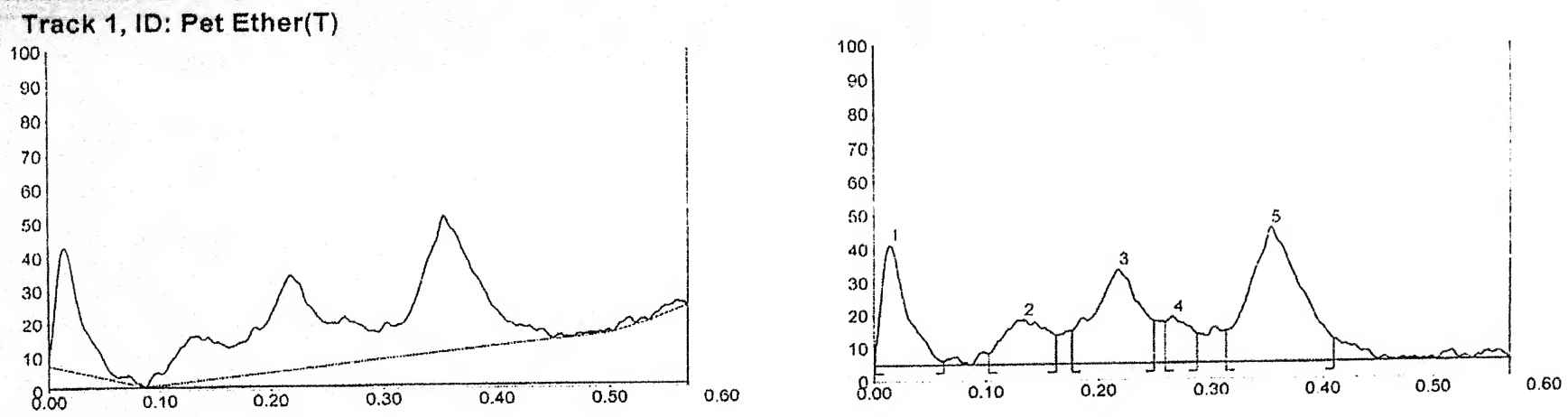
Schematic presentation for HPTLC

TABLE 6: HPTLC PROFILES OF THE SUCCESSIVE SOLVENT EXTRACTS OF FRUITS OF *TRIBULUS TERRESTRIS* LINN.

Solvent system: Tolurene: ethylacetate (8:2) at 366nm

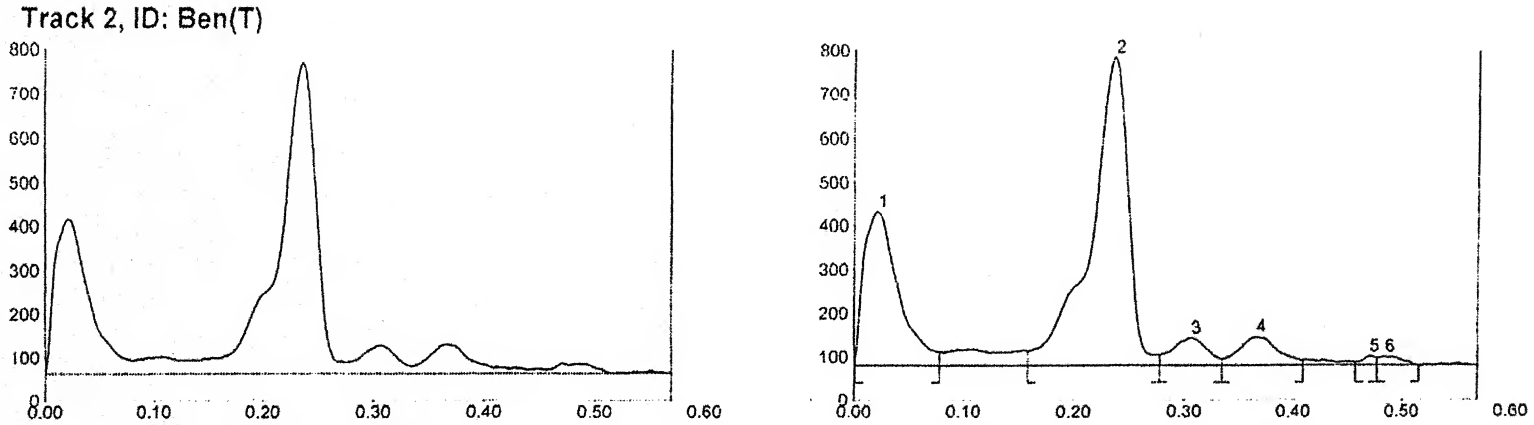
S.No.	Name of the extract	No. of peaks	Rf values	Max. peak height	Percentage peak area
1.	Petroleum ether	1	0.01	72.4	17.33
		2	0.13	26.7	11.22
		3	0.22	57.1	25.02
		4	0.27	27.9	6.41
		5	0.35	81.2	40.03
2.	Benzene	1	0.02	353.0	27.80
		2	0.24	706.0	59.21
		3	0.31	63.7	5.14
		4	0.37	66.5	6.05
		5	0.47	21.1	0.68
		6	0.49	20.1	1.12
3.	Chloroform	1	0.02	176.8	18.37
		2	0.23	501.4	75.31
		3	0.31	17.4	2.12
		4	0.37	31.1	4.21
4.	Ethanol	1	0.03	124.1	26.70
		2	0.24	229.7	65.36
		3	0.31	17.0	3.07
		4	0.38	17.5	4.87
5.	Aqueous	1	0.02	97.9	49.87
		2	0.22	45.6	50.13

Fig. 4: Chromatogram of successive solvent extracts of fruits of *T. terrestris* observed at 366 nm using solvent system Toluene: ethyl acetate (8:2).



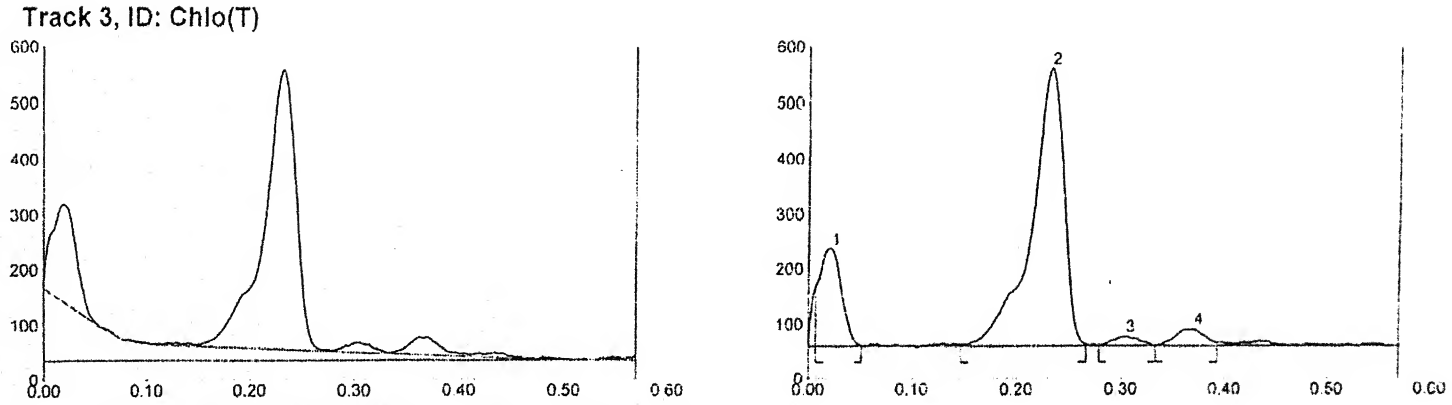
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	7.1	0.01	72.4	27.28	0.06	2.0	1329.5	17.33	Unknown*
2	0.10	6.5	0.13	26.7	10.07	0.16	17.6	860.9	11.22	Unknown*
3	0.18	19.9	0.22	57.1	21.53	0.25	25.8	1919.5	25.02	Unknown*
4	0.26	24.8	0.27	27.9	10.52	0.29	17.1	491.7	6.41	Unknown*
5	0.31	19.2	0.35	81.2	30.59	0.41	13.3	3071.7	40.03	Unknown*

Fig. 5: HPTLC Chromatogram of the petroleum ether extract of fruits of *T. terrestris* scanned at 366 nm using solvent system toluene: ethyl acetate (8:2)



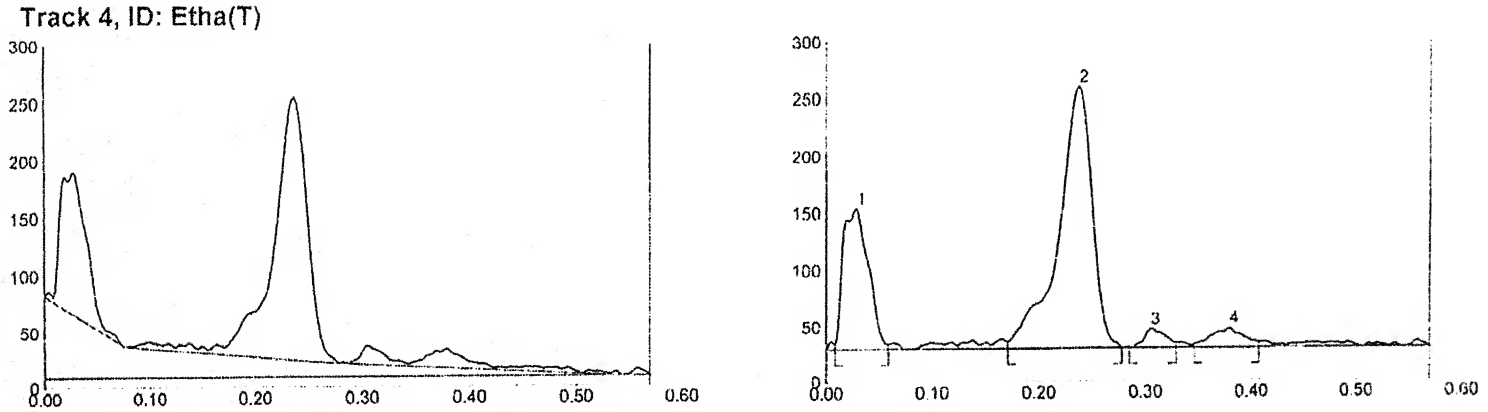
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	15.0	0.02	353.0	28.69	0.08	32.5	9150.1	27.80	unknown *
2	0.16	35.2	0.24	706.0	57.38	0.28	25.2	19488.4	59.21	unknown *
3	0.28	25.2	0.31	63.7	5.18	0.34	15.6	1691.4	5.14	unknown *
4	0.34	15.6	0.37	66.5	5.40	0.41	12.5	1992.0	6.05	unknown *
5	0.46	7.5	0.47	21.1	1.72	0.48	17.8	224.4	0.68	unknown *
6	0.48	17.8	0.49	20.1	1.63	0.52	0.3	367.8	1.12	unknown *

Fig. 6: HPTLC Chromatogram of the benzene extract of the fruits of *T.terrestris* scanned at 366 nm using solvent system toluene: ethyl acetate (8:2)



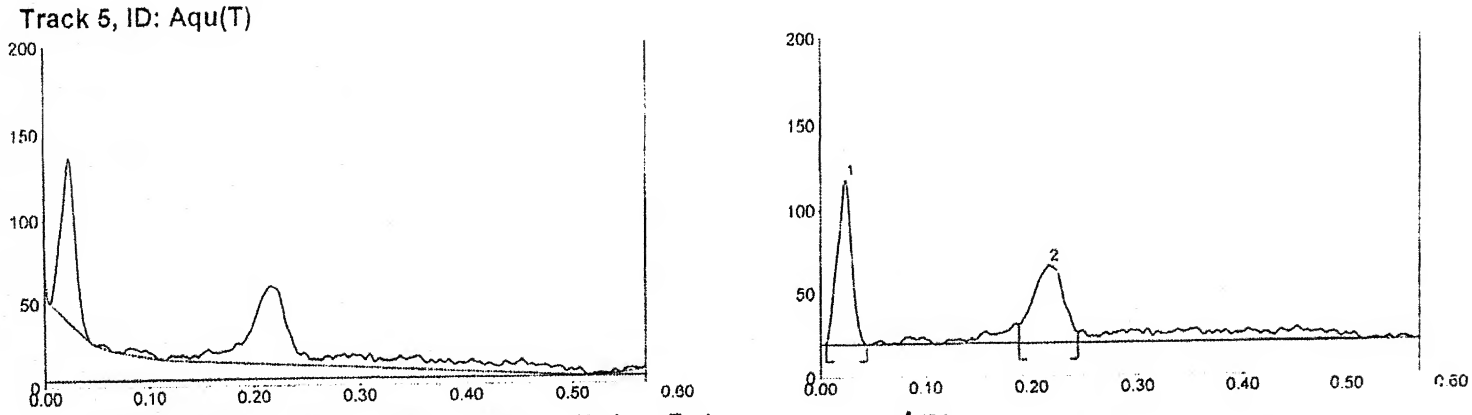
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	108.9	0.02	176.8	24.33	0.05	1.7	3092.8	18.37	unknown *
2	0.15	2.5	0.23	501.4	69.00	0.27	3.5	12680.2	75.31	unknown *
3	0.28	1.2	0.31	17.4	2.39	0.33	0 8	357.3	2.12	unknown *
4	0.33	0.8	0.37	31.1	4.28	0.39	6.0	708.1	4.21	unknown *

Fig. 7: HPTLC Chromatogram of the Chloroform extract of the fruits of *T. terrestris* scanned at 366 nm using solvent system toluene: ethyl acetate (8:2)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	4.0	0.03	124.1	31.95	0.06	4.3	2419.5	26.70	unknown *
2	0.17	6.6	0.24	229.7	59.17	0.28	0.8	5922.2	65.36	unknown *
3	0.29	0.9	0.31	17.0	4.38	0.33	4.5	277.7	3.07	unknown *
4	0.35	3.0	0.38	17.5	4.50	0.41	5.3	440.9	4.87	unknown *

Fig. 8: HPTLC Chromatogram of the ethanol extract of the fruits of *T. terrestris* scanned at 366 nm using solvent system toluene: ehtylacetate (8:2)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.7	0.02	97.9	68.21	0.04	0.0	1082.2	49.87	unknown *
2	0.19	11.0	0.22	45.6	31.79	0.24	5.9	1088.0	50.13	unknown *

Fig. 9: HPTLC Chromatogram of the aqueous extract of the fruits of *T. terrestris* scanned at 366 nm using solvent system toluene: ethyl acetate (8:2)

Discussion:

The proximate analysis of the fruits of *T. terrestris* was carried out to lay certain standards for the air dried drug. The high value of total ash indicated the presence of considerable amount of inorganic constituents in the fruits . The ethanol-soluble extractive and water-soluble extractive values were also rather high , indicated the presence of sugars and resins etc. The qualitative examination of the various solvent extracts of fruits indicated the presence of alkaloids , fixed oils and fats , resins , traces of glycosides , proteins and amino acids , tannins , reducing sugars and sterols and absence of saponins , gums and mucilages . Thin-layer chromatography indicated the presence of diosgenin by Co-chromatography using authentic sample.

The successive solvent extracts of the fruits of *T. terrestris* with petroleum ether, benzene, chloroform, ethanol and water when scanned by HPTLC, using the solvent system toluene: ethyl acetate (8:2) at 366 nm, indicated the presence of 5,6,4,4 and 2 components respectively.

(B) MACROSCOPIC CHARACTERS

The fruit is pedicellate, globose, 1.3 cm in diameter 0.8 cm in thickness, possessing fine woody, densely hairy, spiny cocci. Each coccus possesses two large sharp, pointed, rigid spines directed towards the apex. The other two smaller, shorter spines are directed downwards. Tips of spines almost meet in pairs together forming pentagonal framework around the fruit. Outer surface of the schizocarp is rough. Seeds several in each coccus, with transverse partitions between them.

Color : Yellowish

Odour : faintly aromatic

Taste : slightly acrid (Fig. 10).

(C) MICROSCOPIC CHARACTERS

T.S. of Fruit and Powder Characteristics:

The pericarp is differentiated into epicarp, mesocarp and endocarp. Outer surface of the epicarp is surrounded by non-glandular trichomes. The parenchymatous mesocarp is 6-10 layers thick which embeds calcium oxalate crystals. The sclerenchymatous endocarp is 3-4 layers thick and the cells are compact containing prismatic crystals of calcium oxalate. Fruits were penta locular. Vessels have simple pits and some vessels show helical thickenings. Fibres are lignified, linear long with tapered ends. Transverse section of the fruit and its powder characteristics are shown in Fig.s. 11, 12 and 13.

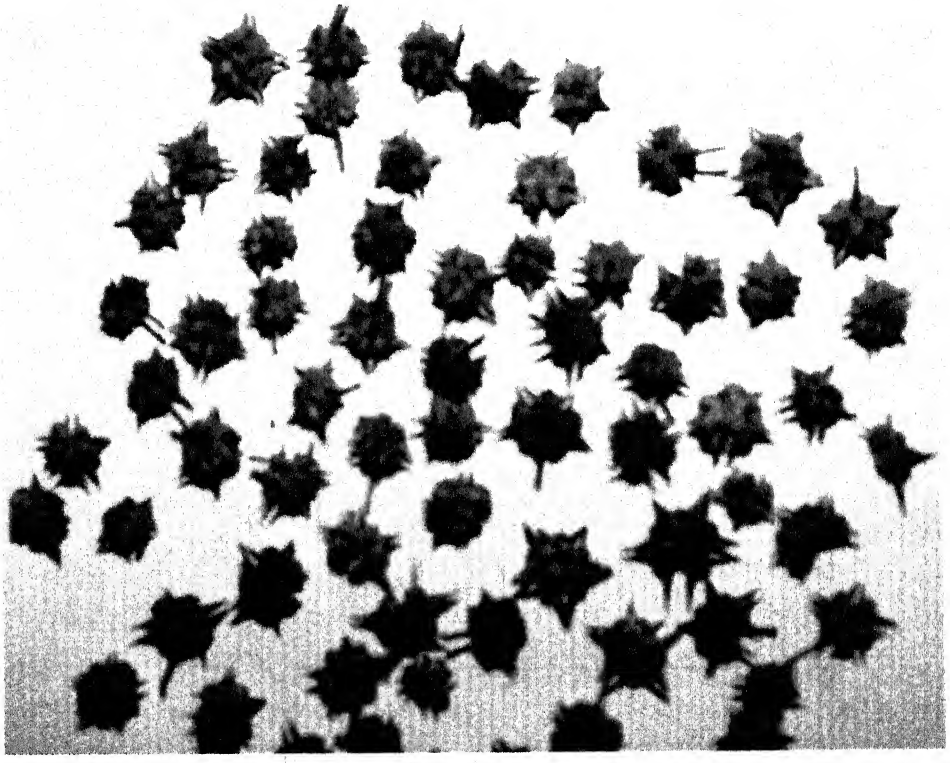


Fig. 10: Fruits of Tribulus terrestris Linn.

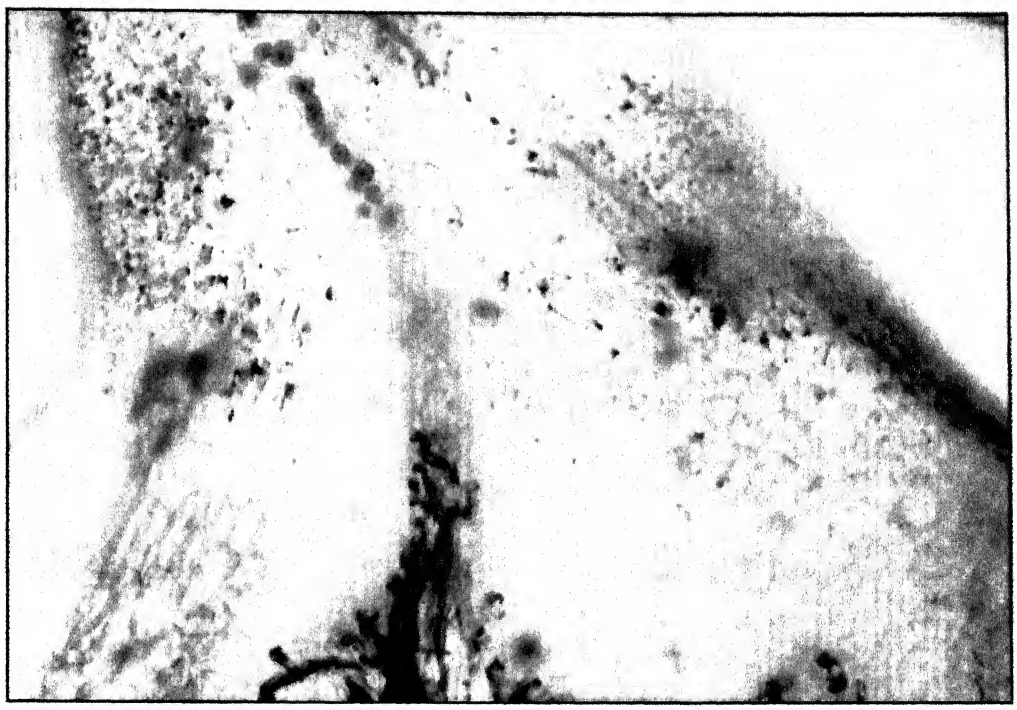


Fig. 12: T.S. of fruit of *T. terrestris* Linn. (Cellular)

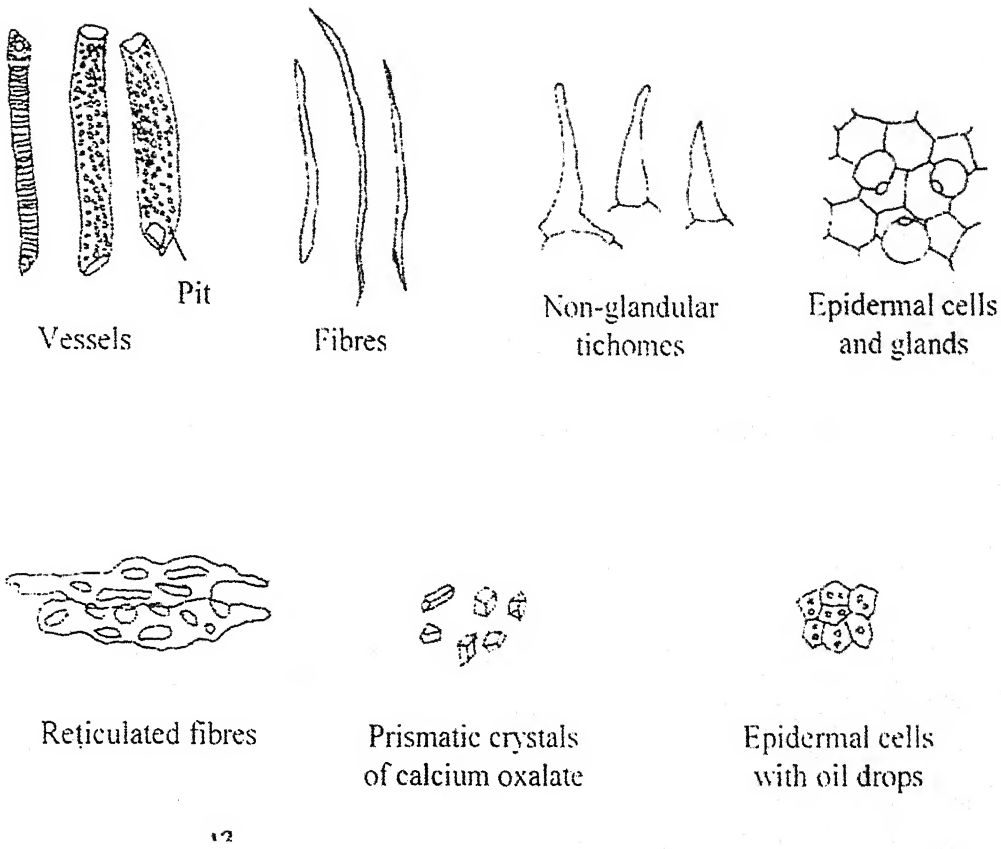


Fig. 13: Powder characteristics of fruit of *T. terrestris* Linn.

(B) STANDARDIZATION OF SEEDS OF *CICHORIUM INTYBUS* LINN.

MATERIALS AND METHODS

The seeds of *Cichorium intybus* were procured from local market of Modinagar, Ghaziabad and were identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun. The seeds were shade dried.

(A) PHYTOCHEMICAL STUDY

1. Quality Parameters

Moderately coarse powders of the seeds were prepared by grinding the seeds in an electric grinder for proximate analysis. Various quality parameters were determined as per I.P. methods as mentioned earlier and results obtained are tabulated in Table 7.

TABLE 7: QUALITY PARAMETERS OF SEEDS OF *CICHORIUM INTYBUS* LINN.

S.No.	Quality parameters	Value
1.	Foreign organic matter	0.67%
2.	Loss on drying	9.11%
3.	Total ash	13.03%
4.	Acid-insoluble ash	1.90%
5.	Sulphated ash	12.33%
6.	Water-soluble ash	2.61%
7.	Ethanol-soluble extractive	1.04%
8.	Water-soluble extractive	2.25%

9.	Petroleum ether-soluble extractive	4.18%
10.	Chloroform-soluble extractive	0.98%
11.	Volatile oil	Nil

2. Fluorescent analysis

Very faint fluorescence in the alcoholic extract at short (254 nm) and long (366 nm) ultra-violet wave lengths was observed as shown in Table 8.

TABLE 8: FLUORESCENT ANALYSIS OF ALCOHOLIC EXTRACT OF SEEDS OF *CICHORIUM INTYBUS* LINN.

S.No.	Light source	Wave length (nm)	Color observed
1.	Ultra-violet light	254	Very Faint
		366	-do-
2.	Ordinary light	-	Colorless

3. Behaviour of powdered drug with different reagents

The behaviour of the seeds powder of C. intybus with different chemical reagents was observed as shown in Table 9.

TABLE 9: BEHAVIOUR OF THE SEEDS POWDER OF *CICHORIUM INTYBUS* LINN. WITH DIFFERENT REAGENTS

S. No.	Reagents	Observation
1	Water	Light graysih brown coloured turbid soln.
2	5% KOH	-do-
3	Dil. HCL	Clean soln.
4	Dil. H_2SO_4	- do-
5	Dil. HNO_3	-do-
6	$FeCl_3$ soln.	Clear orange liquid
7	Dragendorff's soln.	- do-
8	KI and 1 soln	Raddish brown clear liquid

4. Phytochemical Screening:

The seeds of *Cichorium intybus* (150 g) were coarsely powdered and was successively extracted with petroleum ether (60-80°), ethanol and water is a Soxhlet extractor. The various extracts obtained were then subjected to qualitative tests for the presence of important plant constituents like alkaloids , carbohydrates, glycosides, phytosterols, saponins, tannins, proteins and amino acids, fixed oils and fats , gums and mucilages, and resins as described earlier[75].

The tests applied and the results obtained from the tests are shown in Table 10.

TABLE 10: TESTS FOR COMMON PLANT CONSTITUENTS IN VARIOUS EXTRACTS OF SEEDS OF *CICHORIUM INTYBUS* LINN.

S.No.	Plant constituents / Test/Reagent used	Extracts		
		Petroleum ether (60-80°)	Alcohol	Water
1.	**Alkaloids**			
	a) Mayer's reagent	–	–	–
	b) Dragendorff's reagent	–	–	–
	c) Hager's reagent	–	–	–
	d) Wagner's reagent	–	–	–
2.	**Carbohydrates**			
	a) Molisch's reagent	–	+	+
	b) Fehling's solution	–	+	+
3.	**Glycosides**			
	a) Legal's test	–	–	–
	b) Borntrager's test	–	–	–

4.	**Phytosterols**			
	a) Liebermann's test	+	–	–
	b) Liebermann-Burchard's test	+	+	–
5.	**Saponins**			
	Foam test	–	–	–
6.	**Tannins**			
	a) Ferric chloride solution	–	+	+
	b) Lead acetate solution	–	+	–
7.	**Proteins and amino acids**			
	a) Millon's reagent	–	+	+
	b) Ninhydrin reagent	–	+	+
8.	**Fixed oils and fats**			
	a) Spot test	+	–	–
	b) Saponification test	+	–	–
9.	**Gums and mucilages**			
	Alcoholic precipitation	–	–	–
10.	**Resins**	–	–	–

5. Chromatographic analysis:

(A) Thin-Layer Chromatography

140 g seeds powder was extracted with ethyl alcohol in a Soxhlet extractor for 18 h and concentrated under reduced pressure at low temperature (45-50°). The extract was subjected to thin-layer chromatography. Several solvent systems were

tried but the best separation was achieved by the solvent system, Chloroform: methanol: formamide (80: 19: 1). The plate was dried in an oven at 110° for 15 min and the spots were seen in U.V. light then the plate was sprayed with concentrated sulphuric acid followed by drying at 75° for 3 min and the spots were observed in diffused light[79].

The *Rf*-values are recorded in Table 11. The substances corresponding to the spots shown in the chromatoplate were tested also by Liebermann Burchard and Molisch's reagents after being eluted from the plate.

Three spots having the *Rf*-values 0.83, 0.86 and 0.90 gave positive Liebermann Burchard test and other three spots, having the *Rf*-values 0.36, 0.05 and zero showed pale blue , pale blue and green fluorescence respectively in U.V. light , gave positive Molisch's test.

TABLE 11: TLC OF ALCOHOLIC EXTRACT OF SEEDS OF *CICHORIUM INTYBUS* AND RESULTS OBTAINED BY DIFFERENT REAGENTS

S.No. of spots	***Rf* values**	**U.V. Light**	**Sulphuric acid**	**Liebermann Burchard reagent**	**Molisch's reagent**
1.	0.98	–	Violet blue	–	–
2.	0.90	–	Violet	+	–
3.	0.86	–	Blue	+	–
4.	0.83	–	Purple	+	–
5.	0.73	Violet	–	–	–
6.	0.66	–	Red	–	–
7.	0.60	–	Blue	–	–
8.	0.47	–	Pale Violet	–	–
9.	0.36	Pale blue	Dirty green with violet tinge	–	+
10.	0.05	Pale blue		–	+
11.	Zero	Green		–	+

(B) High Performance Thin Layer Chromatography

The seeds of with *C.intybus* were coarsely powdered and was successively extracted with petroleum ether (60-80%), benzene, chloroform, ethanol and water in a Soxhlet extractor. The various extracts obtained were then subjected to HPTLC as mentioned earlier.

4 µl sample of the above each extract was spotted in duplicate on precoated silica gel $G60F_{254}$ TLC plates. The plate was developed using Chloroform: methanol: formamide (8.0:1.9:0.1) as mobile phase and observed under UV light as shown in Figs. 14 and 15. The HPTLC Chromatograms are shown in Fig. 16 to 20 and the number of components separated, their Rf values and percentage peak are tabulated in Table 12.

TABLE 12: HPTLC PROFILES OF THE SUCCESSIVE SOLVENT EXTRACTS OF SEEDS OF *CICHORIUM INTYBUS* LINN.

Solvent system: Chloroform: methanol: formamide (8.0:1.9:0.1)

S.No.	Name of the extract	No. of peaks	Rf values	Max. peak height	Percentage peak area
1.	Petroleum ether	1	0.06	27.3	7.54
		2	0.85	99.9	55.49
		3	0.92	92.6	36.98
2.	Benzene	1	0.03	49.3	28.90
		2	0.70	18.0	17.43
		3	0.93	77.3	53.67
3.	Chloroform	1	0.02	139.3	66.97
		2	0.72	31.8	16.88
		3	0.92	64.7	16.15

4.	Ethanol	1	0.05	689.2	46.15
		2	0.16	325.7	15.82
		3	0.22	168.3	5.48
		4	0.32	186.4	8.95
		5	0.36	184.4	6.36
		6	0.42	120.9	7.30
		7	0.55	51.6	1.65
		8	0.64	46.1	1.03
		9	0.70	52.6	2.36
		10	0.84	117.7	4.14
		11	0.90	42.8	0.75
5.	Aqueous	1	0.02	699.0	61.09
		2	0.09	159.6	18.32
		3	0.15	74.1	5.81
		4	0.20	31.9	2.10
		5	0.28	43.6	3.53
		6	0.31	26.8	1.88
		7	0.40	28.2	2.92
		8	0.56	10.5	0.79
		9	0.79	40.4	2.45
		10	0.85	16.3	1.12

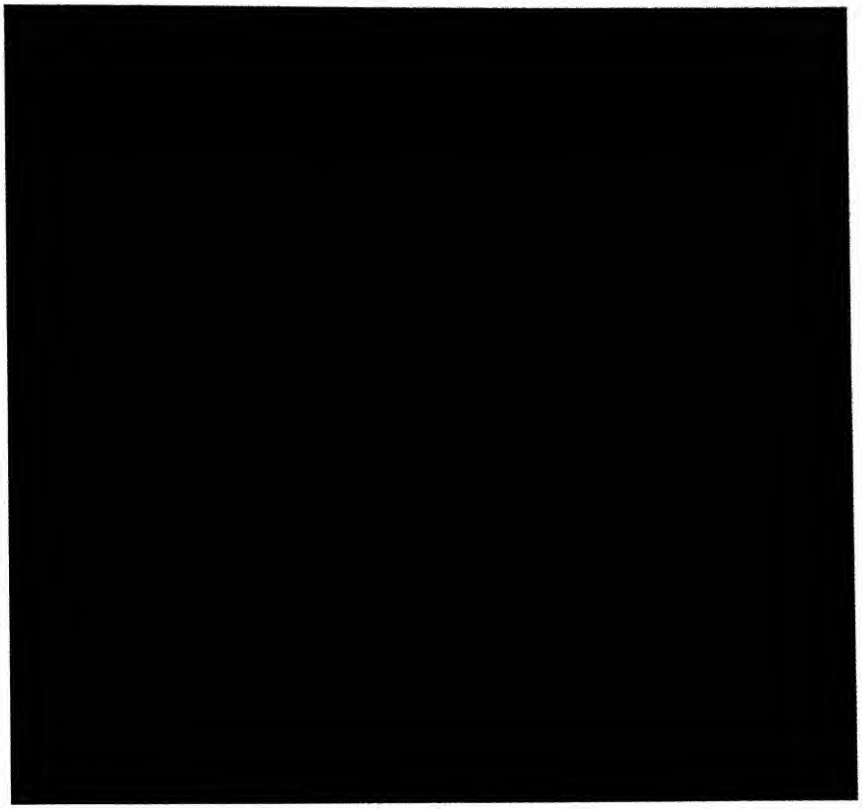
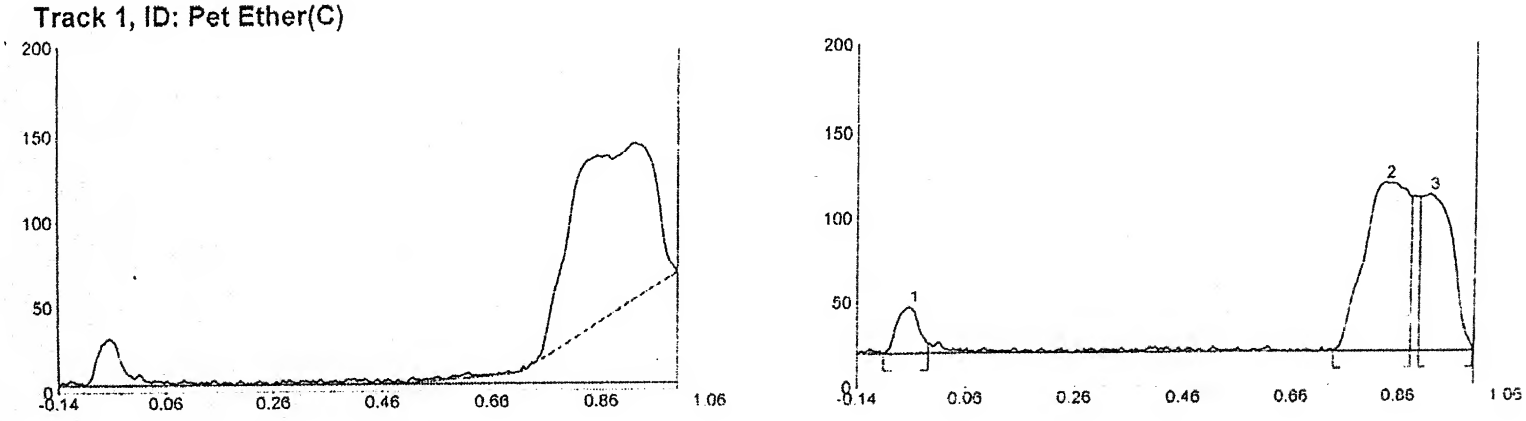


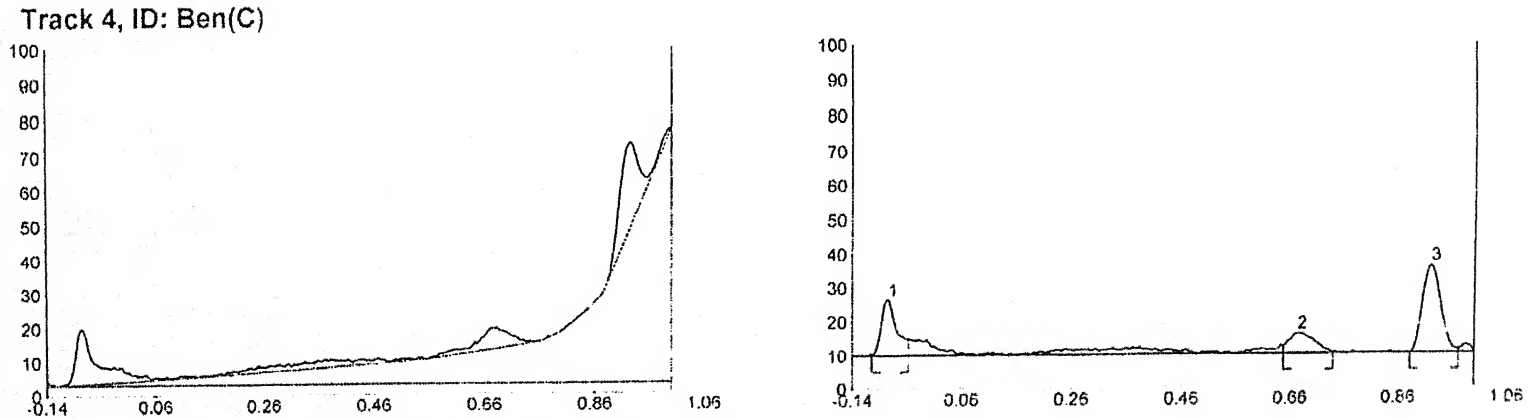
Fig. 14: Chromatogram of petroleum ether, benzene and chloroform extracts of seeds of C. *intybus* observed at 366 nm using solvent system chloroform: methanol: formamide (8.0:1.9:0.1)

Fig. 15: Chromatogram of ethanol and aqueous extracts of seeds of *C. intybus* observed at 244 nm using solvent system Chloroform: methanol: formamide (8.0:1.9: 0.1).



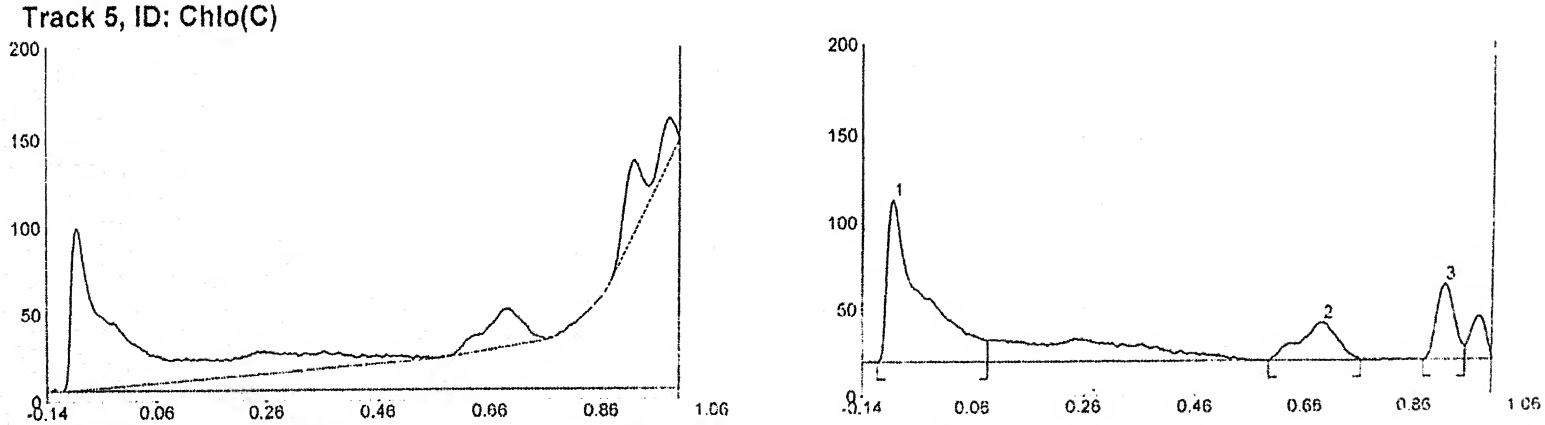
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	02	0.06	27.3	12.43	0.09	6.0	810.9	7.54	Unknown*
2	0.76	0.6	0.85	99.9	45.44	0.89	90.9	5970.6	55.49	Unknown*
3	0.91	90.9	0.92	92.6	42.13	1.00	1.9	3978.6	36.98	Unknown*

Fig. 16: HPTLC Chromatogram of the petroleum ether extract of seeds of *C. intybus* scanned at 366 nm using solvent system Chloroform: methanol: formamide (8.0:1.9:0.1)



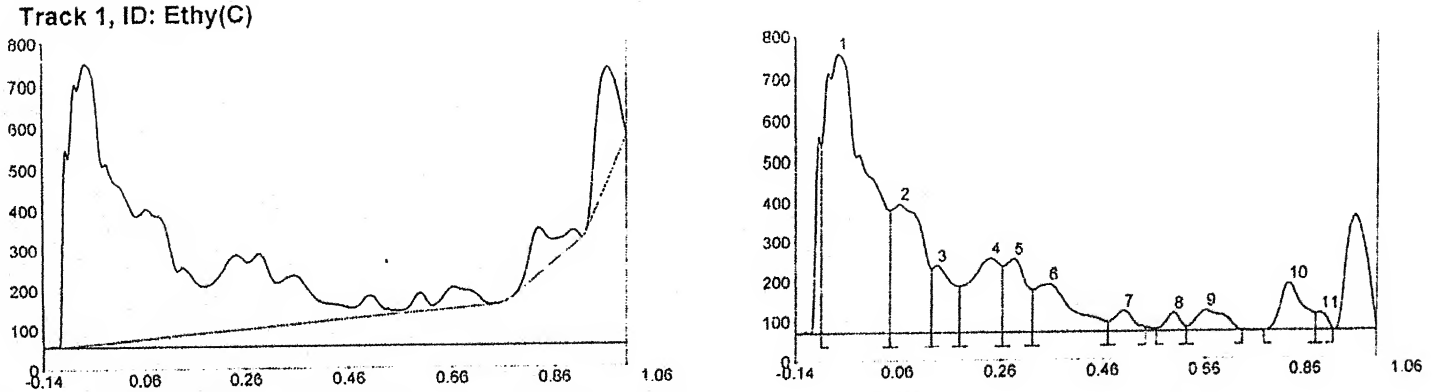
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	1.4	0.03	49.3	34.08	0.06	14.3	1041.0	28.90	unknown *
2	0.68	6.8	0.70	18.0	12.44	0.76	1.1	628.0	17.43	unknown *
3	0.89	0.0	0.93	77.3	53.49	0.97	3.6	1933.5	53.67	unknown *

Fig. 17: HPTLC Chromatogram of the benzene extract of seeds of *C. intybus* scanned at 366 nm using solvent system chloroform: methanol: formamide (8.0:1.9:0.1)



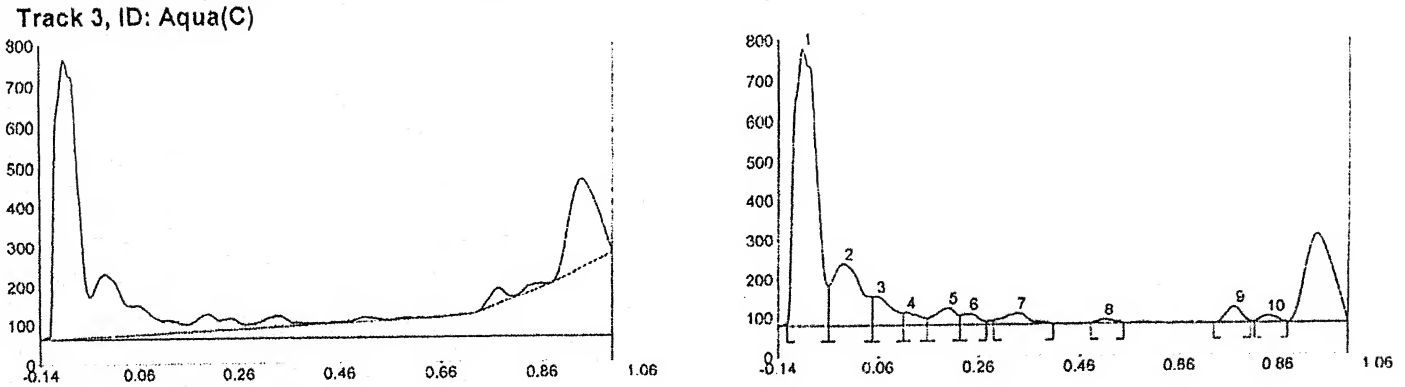
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.00	0.1	0.02	139.3	59.09	0.18	18.3	6479.7	66.97	unknown *
2	0.63	0.4	0.72	31.8	13.48	0.78	0.3	1632.8	16.88	unknown *
3	0.89	0.1	0.92	64.7	27.43	0.96	12.0	1562.9	16.15	unknown *

Fig. 18: HPTLC Chromatogram of the chloroform extract of seeds of *C. intybus* scanned at 366 nm using solvent system chloroform: methanol: formamide (8.0:1.9:0.1)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	467.5	0.05	689.2	34.70	0.14	310.0	42482.4	46.15	unknown *
2	0.14	310.0	0.16	325.7	16.40	0.21	160.9	14562.8	15.82	unknown *
3	0.21	160.9	0.22	168.3	8.48	0.26	117.1	5047.3	5.48	unknown *
4	0.26	117.1	0.32	186.4	9.39	0.34	166.0	8242.3	8.95	unknown *
5	0.34	166.0	0.36	184.4	9.29	0.39	107.8	5854.7	6.36	unknown *
6	0.39	107.8	0.42	120.9	6.09	0.52	25.7	6719.3	7.30	unknown *
7	0.52	25.7	0.55	51.6	2.60	0.59	9.1	1521.6	1.65	unknown *
8	0.61	7.4	0.64	46.1	2.32	0.66	11.4	944.7	1.03	unknown *
9	0.66	11.4	0.70	52.6	2.65	0.76	1.6	2175.8	2.36	unknown *
10	0.80	0.0	0.84	117.7	5.93	0.89	41.0	3814.2	4.14	unknown *
11	0.89	41.0	0.90	42.8	2.15	0.92	0.3	691.9	0.75	unknown *

Fig. 19: HPTLC Chromatogram of the ethanol extract of seeds of *C. intybus* scanned at 254 nm using solvent system chloroform: methanol: formamide (8.0:1.9:0.1)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	2.6	0.02	699.0	61.83	0.06	104.2	22534.1	61.09	unknown *
2	0.06	104.2	0.09	159.6	14.11	0.14	73.0	6758.4	18.32	unknown *
3	0.14	73.0	0.15	74.1	6.56	0.20	30.7	2144.2	5.81	unknown *
4	0.20	30.7	0.20	31.9	2.83	0.24	16.3	773.8	2.10	unknown *
5	0.24	16.3	0.28	43.6	3.86	0.30	22.5	1302.9	3.53	unknown *
6	0.30	22.5	0.31	26.8	2.37	0.35	8.2	692.1	1.88	unknown *
7	0.36	10.4	0.40	28.2	2.49	0.47	1.6	1077.3	2.92	unknown *
8	0.53	2.4	0.56	10.5	0.93	0.59	2.2	290.5	0.79	unknown *
9	0.76	0.0	0.79	40.4	3.57	0.82	3.8	902.4	2.45	unknown *
10	0.83	3.6	0.85	16.3	1.45	0.89	0.2	411.5	1.12	unknown *

Fig. 20: HPTLC Chromatogram of the aqueous extract of seeds of *C. intybus* scanned at 254 nm using solvent system Chloroform: methanol: formamide (8.0:1.9:0.1)

Discussion:

The proximate analysis of the seeds of *C. intybus* was carried out to lay certain standards for the air dried drug. The high value of total ash indicated the presence of considerable amount of inorganic constituents in the seeds.

The petroleum ether-soluble extractive value was also rather than high indicated the presence of fixed oil and fat and sterols etc., which was also observed by phytochemical tests. The phytochemical tests also indicated the presence of carbohydrates, tannins and proteins in both alcohol-soluble and water-soluble extracts and absence of alkaloids, glycosides, saponins, gums and mucilages and resins in all the three solvent extracts. Thin-layer chromatography study showed the presence of three different types of sterols and sugars in ethanolic extract.

The successive solvent extracts of the seeds of *C. intybus* with petroleum ether, benzene, chloroform, ethanol and water when scanned by HPTLC, using solvent system chloroform: methanol: formamide (8.0:1.9:0.1) indicated the presence of 3,3,3,11 and 10 components respectively.

(B) MACROSCOPIC CHARACTERS

Colour	:	Light brown to pale brown
Odour	:	Odourless
Size	:	3-4 mm long and 2-3 mm wide
Shape	:	Oval.
Surface	:	Rough
Taste	:	Bland (Fig.. 21).

(C) MICROSCOPIC CHARACTERS

T.S. of Seed and Powder characteristics:

Transverse section of seed shows testa consisting of a single layer of columnar, thin walled, parenchymatous palisade like cells.

Powder is whitish in colour; consisting of following characteristics:

1. Epidermis: These are thick walled, polyhedral to tangentially elongated cells of the testa.
2. Endosperm: These are thickened cell walls with numerous pits.
3. Testa: It is yellow pigment layer associated with pitted cells of the endosperm.
4. Starch grains: These are abundant, simple spherical as well as compound.

Transverse section of the seed and its powder characteristics were observed under microscope as shown in Figs. 22 and 23.

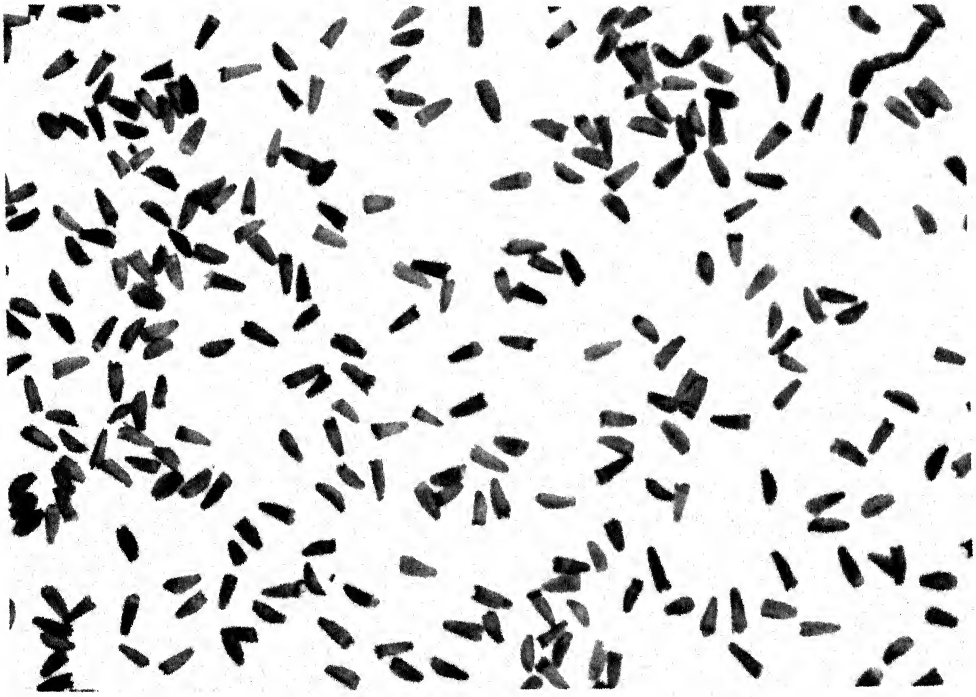


Fig. 21: Seeds of *Cichorium intybus* Linn.

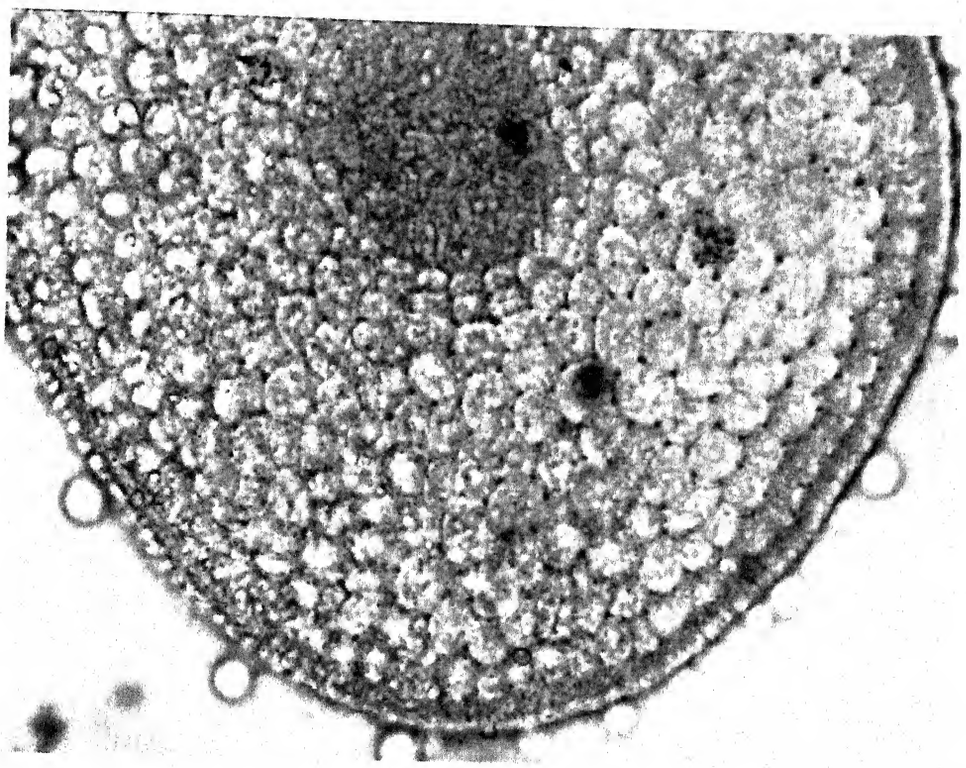


Fig. 22: T.S of seed of *C. intybus* Linn. (Cellular)

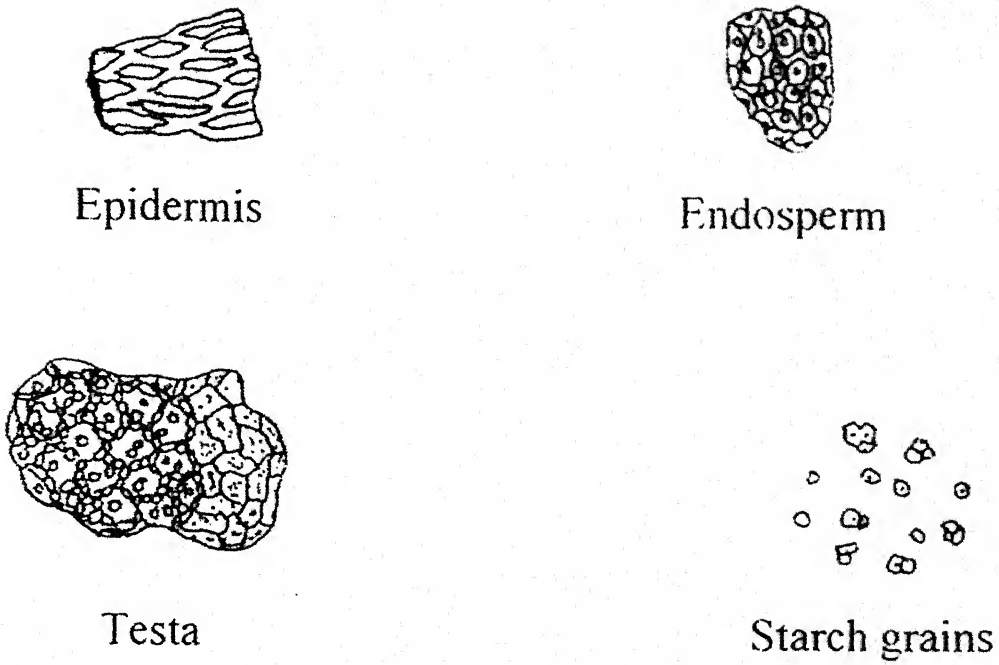


Fig. 23: Powder characteristics of seed of *C. intybus* Linn.

(C) STANDARDIZATION OF SEEDS OF *DOLICHOS BIFLORUS* LINN.

MATERIALS AND METHODS

The seeds of *Dolichos biflorus* were procured from local market of Modinagar, Ghaziabad and were identified by Dr. H.B.Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun. The seeds were shade dried.

(A) PHYTOCHEMICAL STUDY

1. Quality Parameters

Moderately coarse powder of the seeds were prepared by grinding the seeds in an electric grinder for proximate analysis. Various quality parameters were determined as per I.P. methods as mentioned earlier and results obtained are tabulated in Table 13.

TABLE 13: QUALITY PARAMETERS OF SEEDS *DOLICHOS BIFLORUS* LINN.

S.No.	Quality parameters	Value
1.	Foreign organic matter	Nil
2.	Loss on drying	10.9%
3.	Total ash	4.07%
4.	Acid-insoluble ash	0.80%
5.	Sulphated ash	8.38%
6.	Water-soluble ash	2.97%
7.	Ethanol-soluble extractive	0.48%
8.	Water-soluble extractive	5.16%

9.	Petroleum ether-soluble extractive	1.56%
10.	Chloroform-soluble extractive	0.23%
11.	Volatile oil	Nil

2. Fluorescent analysis

Very faint fluorescence in the alcoholic extract at short (254 nm) and long (366 nm) ultra-violet wavelengths was observed as shown in Table 14 .

TABLE 14 : FLUORESCENT ANALYSIS OF ALCOHOLIC EXTRACT OF SEEDS OF *DOLICHOS BIFLORUS* LINN.

S. No.	Light Source	Wave length (nm)	Color observed
1.	Ultra - violet light	254	Very Faint
		366	-do-
2.	Ordinary light	-	Colorless

3. Behaviour of powdered drug with different reagents

The behaviour of the seeds powder of *D.biflorus* with different chemical reagents was observed as shown in Table 15.

TABLE 15: BEHAVIOUR OF THE SEEDS POWDER OF *DOLICHOS BIFLORUS* LINN. WITH DIFFERENT REAGENTS.

S.No	Reagents	Observation
1.	Water	Dark grayish brown coloured turbid solution
2.	5% KOH	Greenish coloured turbid solution
3.	Dil. Hcl	Clear solution
4.	Dil. H_2So_4	- do-

5.	Dil. HNO3	- do -
6.	$FeCl_3$ Soln.	Clear orange liquid
7.	Dragendorff's soln.	- do-
8.	KI and I soln.	Reddish brown clear liquid

4. Phytochemical Screening:

The seeds of *Dolichos biflorus* (350g) were coarsely powdered and was successively extracted with petroleum ether (60-80°), ethanol and water in a Soxhlet extractor. The various extracts obtained were subjected to qualitative tests for the presence of important plant constituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins and amino acids, fixed oils and fats, gums and mucilages, and resins as described earlier[75].

The results obtained from the tests are shown in Table 16.

TABLE 16: TESTS FOR COMMON PLANT CONSTITUENTS IN VARIOUS EXTRACTS OF SEEDS OF *DOLICHOS BIFLORUS* LINN.

S.No.	Plant Constituents Test/Reagent used	Extracts		
		Petroleum ether (60-80°)	Alcohol	Water
1.	**Alkaloids**			
	a) Mayer's reagent	–	–	–
	b) Dragendorff's reagent	–	–	–
	c) Hager's reagent	–	–	–
	d) Wagner's reagent	–	–	–

2.	**Carbohydrates**			
	a) Molisch's reagent	–	+	+
	b) Fehling's solution	–	+	+
3.	**Glycosides**			
	a) Legal's test	–	–	–
	b) Borntrager's test	–	–	–
4.	**Phytosterols**			
	a) Liebermann's test	+	–	–
	b) Liebermann-Burchard's test	+	+	–
5.	**Saponins**			
	Foam test	–	–	–
6.	**Tannins**			
	a) Ferric Chloride solution	–	–	–
	b) Lead acetate solution	–	–	–
7.	**Proteins and amino acids**			
	a) Millon's reagent	–	+	+
	b) Ninhydrin reagent	–	+	+
8.	**Fixed oils and fats**			
	a) Spot test	+	–	–
	b) Saponification test	+	–	–
9.	**Gums and mucilages**			
	Alcoholic precipitation	–	–	–
10.	**Resins**	–	–	–

5. Chromatographic analysis:

(A) Thin Layer Chromatography

Amino acids

Seeds powder was defatted with petroleum ether (60-80°) in Soxhlet extractor. 1.0 g of defatted seed powder was warmed with 10 ml ethanol (70% v/v) for 30 min and centrifuged. The residue was re-extracted with ethanol and centrifuged. This process was repeated 8 to 9 times till the supernatant was negative to ninhydrin test. All the supernatants were combined and evaporated to dryness in vacuo. The residue was dissolved in 0.5-1.0 ml distilled water and centrifuged. The clear supernatant was subjected to thin-layer chromatography using n-Butanol: acetic acid: water (8: 2: 2) and 96% Ethanol: water (7: 3) as mobile phase. The chromatograms were sprayed with ninhydrin (0.1%w/v) in butanol[80].

The *Rf*-values are recorded in Table 17.

TABLE 17: TLC OF AMINO ACIDS OF SEEDS OF *DOLICHOS BIFLORUS* USING VARIOUS SOLVENTS

S.No.	n-Butanol:acetic acid: water (8:2:2)		96% Ethanol:water (7:3)		Amino acids identified
	Rf reported	*Rf* found	*Rf* reported	*Rf* found	
1.	0.22	0.22	–	–	Alanine
2.	0.05	0.06	0.33	0.33	Histidine
3.	0.09	0.09	0.39	0.39	Cystine
4.	0.17	0.17	0.55	0.55	Aspartic acid
5.	0.44	0.45	0.61	0.60	Leucine
6.	0.18	0.18	0.43	0.42	Glycine
7.	–	–	0.48	0.48	Serine
8.	0.03	0.03	0.03	0.03	Lysine

Carbohydrates

The defatted seeds were extracted with water and concentrated to dark brown mass. It was found to respond to positive tests for sugars which were identified by thin-layer chromatography on silica gel G , impregnated with sodium acetate buffer using Chloroform : methanol (6 : 4) and Acetone : water (9 : 1) as solvent system . The chromatograms were sprayed with aniline hydrogen phthalate as spraying reagent[81].

The *Rf*-values are recorded in Table 18.

TABLE 18: TLC OF CARBOHYDRATES OF SEEDS OF *DOLICHOS BIFLORUS* USING VARIOUS SOLVENTS

S.No.	Chloroform:methanol (6:4)		Acetone:water (9:1)		Sugars Identified
	Rf reported	*Rf* found	*Rf* reported	*Rf* found	
1.	0.54	0.53	0.71	0.72	Rhamnose
2.	0.41	0.41	0.53	0.53	Arabinose
3.	0.30	0.29	0.47	0.48	Fructose
4.	0.27	0.27	0.45	0.45	Galactose
5.	0.37	0.36	0.55	0.56	Glucose

(B) High Performance Thin Layer Chromatography

The seeds of *D. biflorus* were coarsely powdered and was successively extracted with petroleum ether ($60\text{-}80^0$), benzene, chloroform, ethanol and water in a Soxhlet extractor. The various extracts obtained were subjected to HPTLC as mentioned earlier.

Amino acids

4µl sample of the above each extract was spotted in duplicate along with solution of 8 authentic aminoacids viz., alanine, histidine, cystine, aspartic acid, leucine, glycine, serine and lysine on precoated silica gel $G60F_{254}$ TLC plate. The plates were developed using n-butanol: acetic acid: water (8:2:2) and 96% ethanol: water (7:3) as mobile phases and observed under UV light as shown in Figs. 24 to 27. The HPTLC Chromatograms are shown in Figs. 34 to 42 and the number of components separated, their Rf values and percentage peak area are tabulated in Tables 19a and 19b.

Carbohydrates

The above extracts were again spotted along with solution of 5 authentic sugars viz., rhamnose, arabinose, fructose, galactose and glucose on precoated silica gel $G60F_{254}$ TLC plate. The plates were developed using chloroform: methanol (6:4) and acetone: water (9:1) as mobile phases and observed under UV light as shown in Figs. 28 to 33. The HPTLC Chromatograms are shown in Figs. 43 to 54 and the number of components separated, their Rf values and percentage peak area are tabulated in Tables 20a and 20b.

TABLE 19: HPTLC PROFILES OF THE SUCCESSIVE SOLVENT EXTRACTS OF SEEDS OF *DOLICHOS BIFLORUS* LINN.

(a) **Solvent system:** n-butanol: acetic acid : water (8:2:2) at 254 nm

S.No.	Name of the extract	No. of Peaks	Rf values	Max. Peak height	Percentage
1.	Petroleum ether	1	0.24	12.5	0.25
		2	0.42	19.7	0.65
		3	0.44	24.4	0.45
		4	0.45	21.7	0.94
		5	0.65	52.4	3.91
		6	0.77	114.0	12.92
		7	0.91	505.4	80.86
2.	Benzene	1	0.18	12.9	0.34
		2	0.19	19.1	0.36
		3	0.22	34.5	1.70
		4	0.32	11.3	0.41
		5	0.37	10.1	0.14
		6	0.44	16.3	0.71
		7	0.52	40.3	2.16
		8	0.57	57.9	2.20
		9	0.63	73.9	3.27
		10	0.75	139.2	14.05
		11	0.89	532.0	74.65

3.	Authentic amino acids	1	0.01	12.0	0.18
		2	0.06	13.1	0.35
		3	0.10	15.1	0.30
		4	0.33	10.4	0.17
		5	0.47	24.8	0.68
		6	0.59	56.0	3.02
		7	0.77	233.0	26.17
		8	0.88	588.4	69.13
4.	Chloroform	1	0.01	77.0	6.05
		2	0.22	15.8	5.43
		3	0.44	90.0	62.63
		4	0.57	45.1	25.88
5	Ethanol	1	0.03	28.1	5.24
		2	0.20	52.4	24.65
		3	0.29	20.1	9.77
		4	0.48	10.7	6.48
		5	0.65	30.3	10.68
		6	0.75	43.8	25.98
		7	0.92	18.4	17.24

(b) **Solvent system:** 96% ethanol: water (7:3)

S.No.	Name of the extract	No. of Peaks	Rf Values	Max. Peak height	Percentage
1.	Chloroform	1	0.00	433.8	71.37
		2	0.87	93.5	20.01
		3	0.90	54.9	8.62
2.	Ethanol	1	0.01	247.0	24.72
		2	0.12	14.6	2.48
		3	0.22	13.3	2.96
		4	0.52	50.8	13.66
		5	0.81	30.7	3.31
		6	0.87	138.9	52.88
3.	Aqueous	1	0.01	124.9	6.58
		2	0.15	108.7	7.02
		3	0.21	101.2	9.05
		4	0.28	79.5	6.59
		5	0.36	73.4	2.04
		6	0.38	66.6	2.96
		7	0.52	87.4	8.34
		8	0.64	18.5	0.82
		9	0.74	45.0	3.74
		10	0.89	321.5	52.86

4.	Authentic amino acids	1	0.00	25.1	8.10
		2	0.10	15.0	2.74
		3	0.13	48.8	10.40
		4	0.20	24.0	4.49
		5	0.28	30.3	8.13
		6	0.32	17.3	3.0
		7	0.38	26.4	4.85
		8	0.63	25.0	6.63
		9	0.76	27.7	4.77
		10	0.85	63.7	27.30
		11	0.89	33.0	14.50
		12	0.95	21.6	5.08

TABLE 20: HPTLC PROFILES OF THE SUCCESSIVE SOLVENT EXTRACTS OF SEEDS OF *DOLICHOS BIFLORUS* LINN.

(a) **Solvent system:** chloroform: methanal (6:4)

S.No.	Name of the extract	No. of Peaks	Rf values	Max. Peak height	Percentage
1.	Petroleum ether	1	0.01	11.9	1.07
		2	0.09	16.2	3.86
		3	0.34	124.1	17.57
		4	0.68	15.5	4.36
		5	0.70	16.0	3.19
		6	0.91	109.9	69.95

2.	Benzene	1	0.01	14.3	0.44
		2	0.08	12.8	0.54
		3	0.12	15.2	0.42
		4	0.15	41.9	1.28
		5	0.22	13.6	0.74
		6	0.30	48.7	1.70
		7	0.34	11.5	0.56
		8	0.52	20.7	1.09
		9	0.68	91.0	3.61
		10	0.75	33.9	2.19
		11	0.77	44.5	2.55
		12	0.85	51.1	4.02
		13	0.92	454.9	80.85
3.	Chloroform	1	0.00	80.1	4.87
		2	0.10	133.1	3.04
		3	0.48	15.0	1.27
		4	0.58	54.6	3.03
		5	0.68	44.2	2.79
		6	0.73	128.0	9.73
		7	0.86	441.7	75.27
4	Ethanol	1	0.00	202.5	3.82
		2	0.05	164.5	1.89
		3	0.12	189.4	6.37
		4	0.17	140.3	2.79

		5	0.34	304.6	7.66
		6	0.38	384.5	12.82
		7	0.54	280.1	6.31
		8	0.61	459.3	17.77
		9	0.69	316.5	7.77
		10	0.75	301.1	9.04
		11	0.90	436.4	23.76
5.	Aqueous	1	0.01	32.8	1.06
		2	0.03	227.0	17.36
		3	0.07	153.1	29.38
		4	0.13	119.1	9.57
		5	0.15	94.3	15.50
		6	0.23	56.8	6.07
		7	0.26	45.8	3.92
		8	0.30	24.5	2.31
		9	0.44	34.2	3.55
		10	0.45	26.9	2.05
		11	0.72	20.5	0.89
		12	0.74	16.7	1.19
		13	0.77	20.6	2.29
		14	0.88	28.8	3.81
		15	0.91	16.8	1.05

6.	Authentic Sugars	1	0.01	241.3	32.69
		2	0.04	275.5	32.80
		3	0.07	27.8	4.27
		4	0.14	19.7	1.34
		5	0.21	157.9	9.49
		6	0.25	16.1	2.13
		7	0.35	16.6	1.95
		8	0.41	15.2	2.18
		9	0.44	13.8	1.24
		10	0.58	15.5	1.90
		11	0.76	16.7	2.62
		12	0.79	19.3	1.97
		13	0.81	12.6	1.03
		14	0.83	15.9	2.77
		15	0.86	16.7	1.61

(b) Solvent system: acetone: water (9:1) at 366 nm

S.No.	Name of the extract	No. of Peaks	Rf values	Max. Peak height	Percentage
1.	Petroleum ether	1	0.03	56.7	2.94
		2	0.06	24.5	1.74
		3	0.09	30.5	1.38
		4	0.11	36.8	1.15
		5	0.17	68.9	3.23
		6	0.24	69.4	6.81

		7	0.27	122.1	8.40
		8	0.38	106.5	5.54
		9	0.42	112.3	4.10
		10	0.43	115.6	4.89
		11	0.47	112.7	4.48
		12	0.53	104.1	8.43
		13	0.67	99.0	10.26
		14	0.76	74.8	3.68
		15	0.84	182.2	26.99
		16	0.88	61.9	1.97
		17	0.89	59.6	4.01
2.	Benzene	1	0.01	178.5	4.53
		2	0.10	81.2	1.37
		3	0.15	48.3	1.79
		4	0.17	52.9	1.71
		5	0.21	65.6	1.27
		6	0.24	67.4	3.00
		7	0.26	71.5	2.26
		8	0.28	82.0	2.57
		9	0.35	110.1	6.11
		10	0.39	108.3	4.41
		11	0.44	110.2	5.47
		12	0.47	109.3	3.13
		13	0.48	107.1	1.94
		14	0.50	127.4	7.05

		15	0.55	99.4	4.28
		16	0.66	107.9	5.54
		17	0.69	109.0	3.46
		18	0.73	70.9	1.25
		19	0.78	99.5	4.65
		20	0.84	430.6	33.24
		21	0.90	25.5	1.0
3.	Chloroform	1	0.01	665.8	63.83
		2	0.07	52.4	3.64
		3	0.10	31.4	1.96
		4	0.11	35.9	2.64
		5	0.15	46.9	4.55
		6	0.19	36.8	2.74
		7	0.38	41.3	5.78
		8	0.49	42.6	6.22
		9	0.83	34.5	5.04
		10	0.93	24.8	3.61
4	Ethanol	1	0.01	34.82	14.86
		2	0.16	96.9	3.50
		3	0.22	106.4	9.37
		4	0.28	116.5	1.92
		5	0.32	240.1	13.94
		6	0.36	149.6	2.79
		7	0.39	105.1	2.21
		8	0.44	110.1	4.70

		9	0.52	121.1	5.83
		10	0.61	111.1	3.61
		11	0.69	239.0	14.70
		12	0.88	218.4	22.56
5.	Aqueous	1	0.01	28.3	0.58
		2	0.04	410.8	21.65
		3	0.10	252.3	16.55
		4	0.15	147.7	7.41
		5	0.24	184.3	23.10
		6	0.31	83.2	2.37
		7	0.33	55.1	2.01
		8	0.41	111.8	10.47
		9	0.49	47.7	2.35
		10	0.54	43.1	0.87
		11	0.57	43.8	1.78
		12	0.61	149.0	2.63
		13	0.64	20.3	0.53
		14	0.72	13.4	0.30
		15	0.75	13.3	0.31
		16	0.78	34.3	1.04
		17	0.81	130.0	2.84
		18	0.87	51.1	3.20

6.	Authentic sugars	1	0.02	27.4	7.77
		2	0.05	29.6	13.63
		3	0.09	24.3	6.75
		4	0.11	21.5	6.15
		5	0.14	70.7	21.39
		6	0.29	17.3	3.41
		7	0.42	25.8	5.38
		8	0.49	14.9	4.87
		9	0.51	15.4	2.29
		10	0.62	14.6	3.34
		11	0.69	23.6	5.93
		12	0.81	13.1	4.34
		13	0.88	24.5	6.48
		14	0.90	42.9	8.26

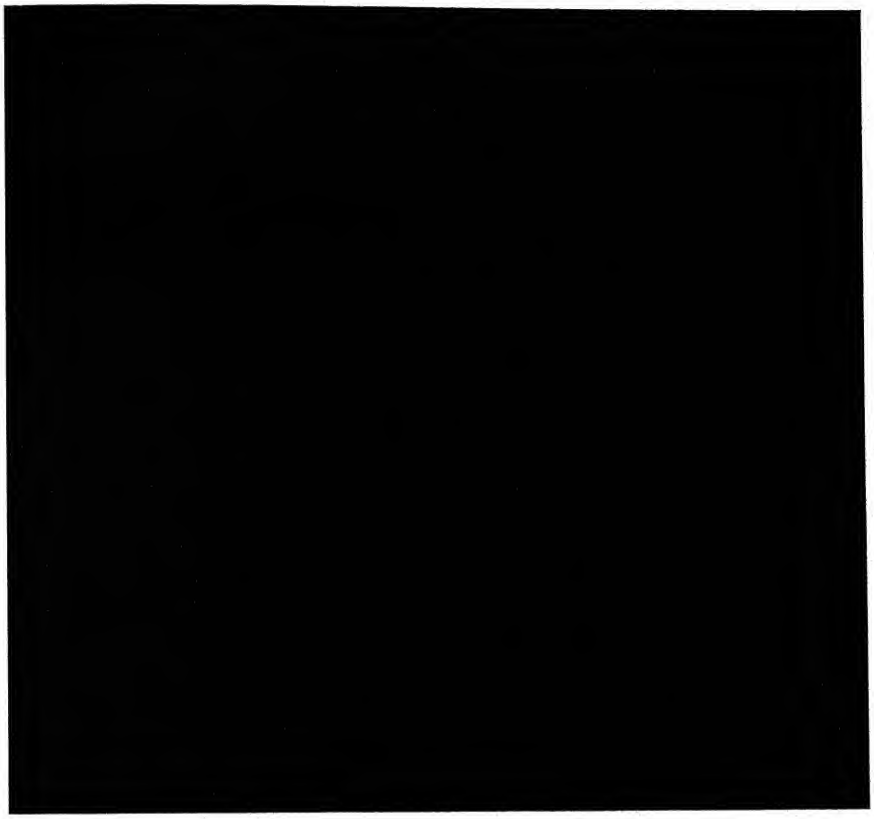


Fig. 24: Chromatogram of Petroleum ether and benzene extracts of seeds of *D.biflorus* along with authentic amino acids observed at 254 nm using solvent system n-butanol: acetic acid: water (8:2:2)

Fig. 25: Chromatogram of chloroform and ethanol extracts of seeds of *D.biflorus* observed at 254 nm using solvent system n-butanol acetic acid: water (8:2:2)

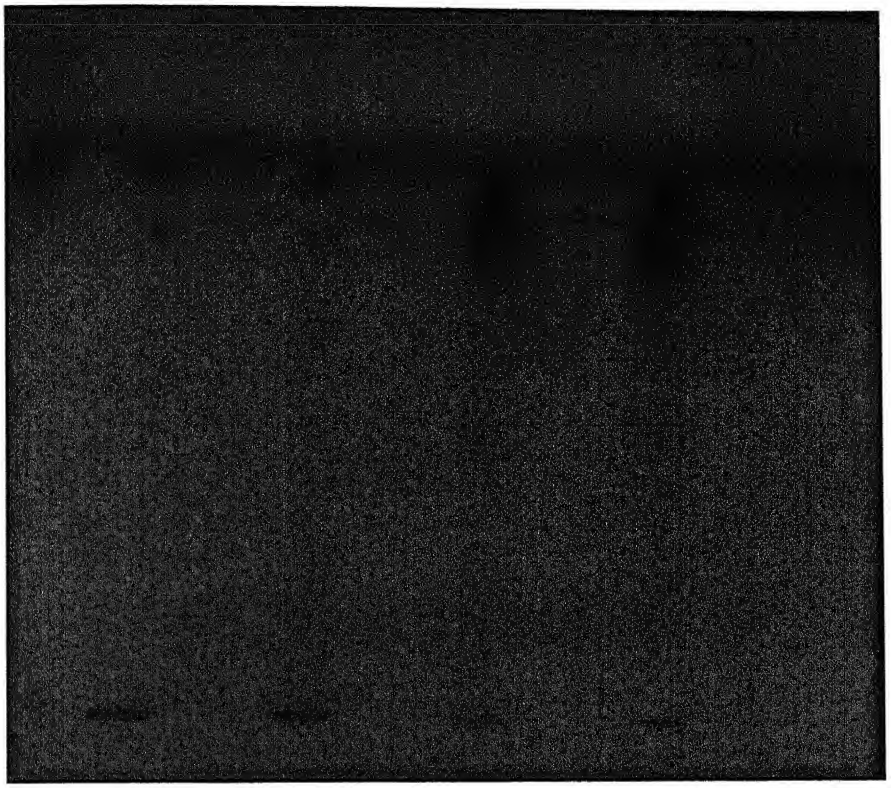


Fig. 26: Chromatogram of Chloroform and ethanol extracts of seeds of *D.biflorus* observed at 254 nm using solvent system 96% ethanol: water (7:3)

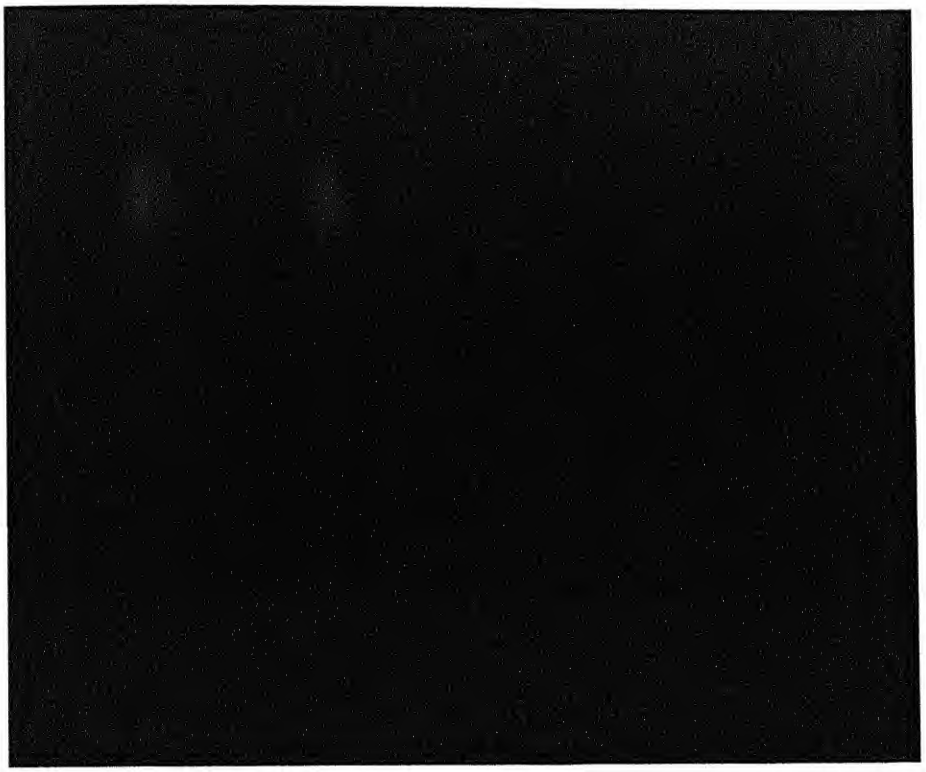


Fig. 27: Chromatogram of aqueous extract of seeds of *D.biflorus* along with authentic amino acids observed at 366 nm using solvent system 96% ethanol: water (7:3)

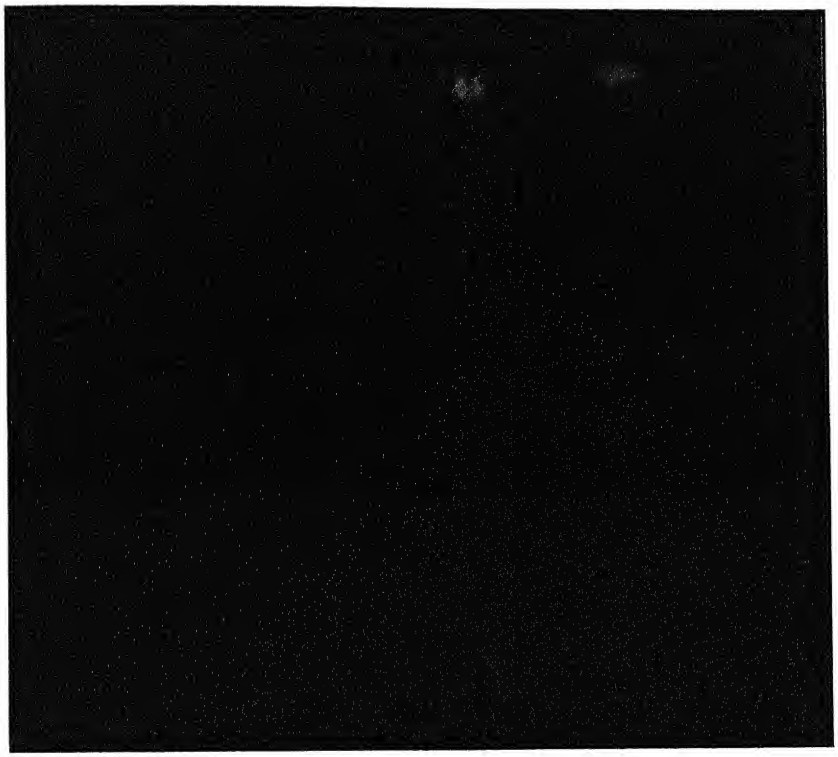


Fig. 28: Chromatogram of Petroleum ether and benzene extracts of seeds of *D.biflorus* observed at 366 nm using solvent system chloroform: methanol (6:4)

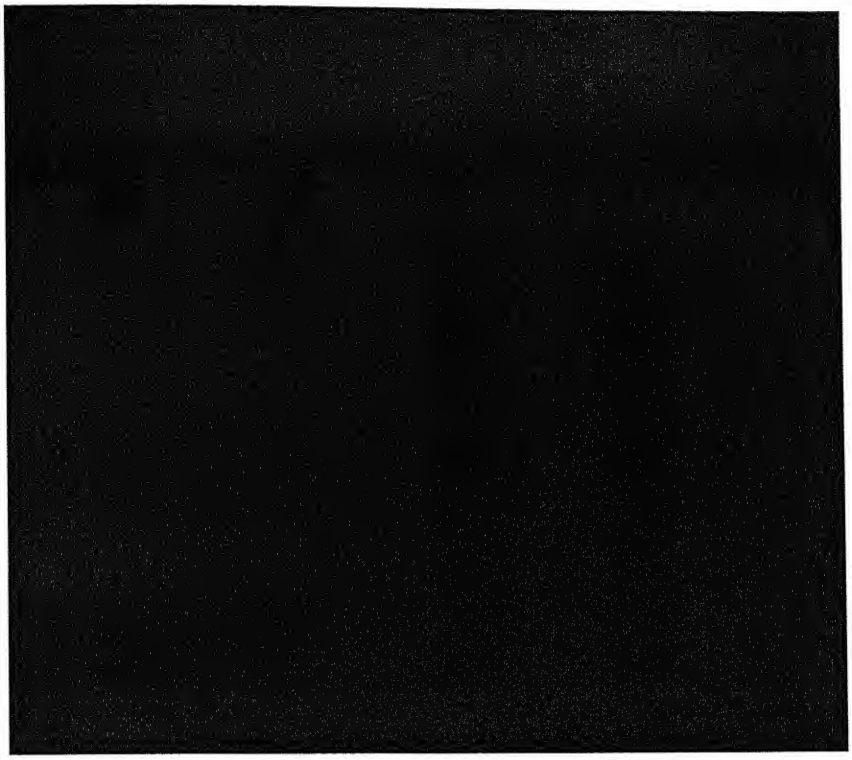


Fig. 29: Chromatogram of chloroform and ethanol extracts of seeds of *D.biflorus* observed at 254 nm using solvent system chloroform: methanol (6:4)

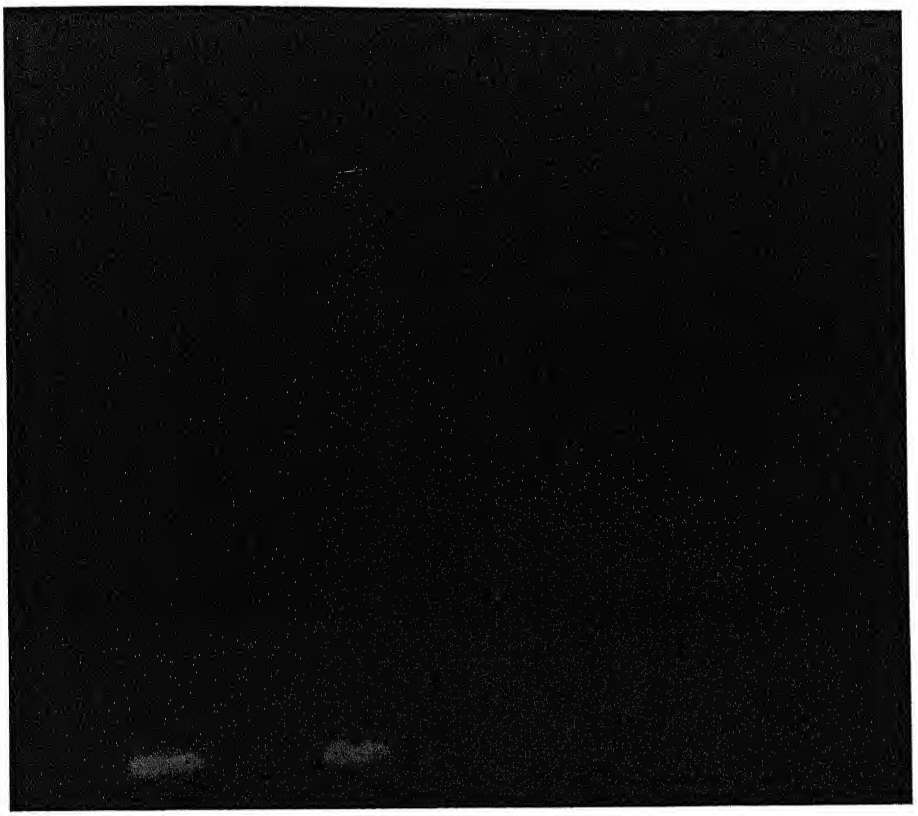


Fig. 30: Chromatogram of aqueous extract of seeds of D. biflorus along with authentic sugars observed at 366 nm using solvent system chloroform: methanol (6:4)

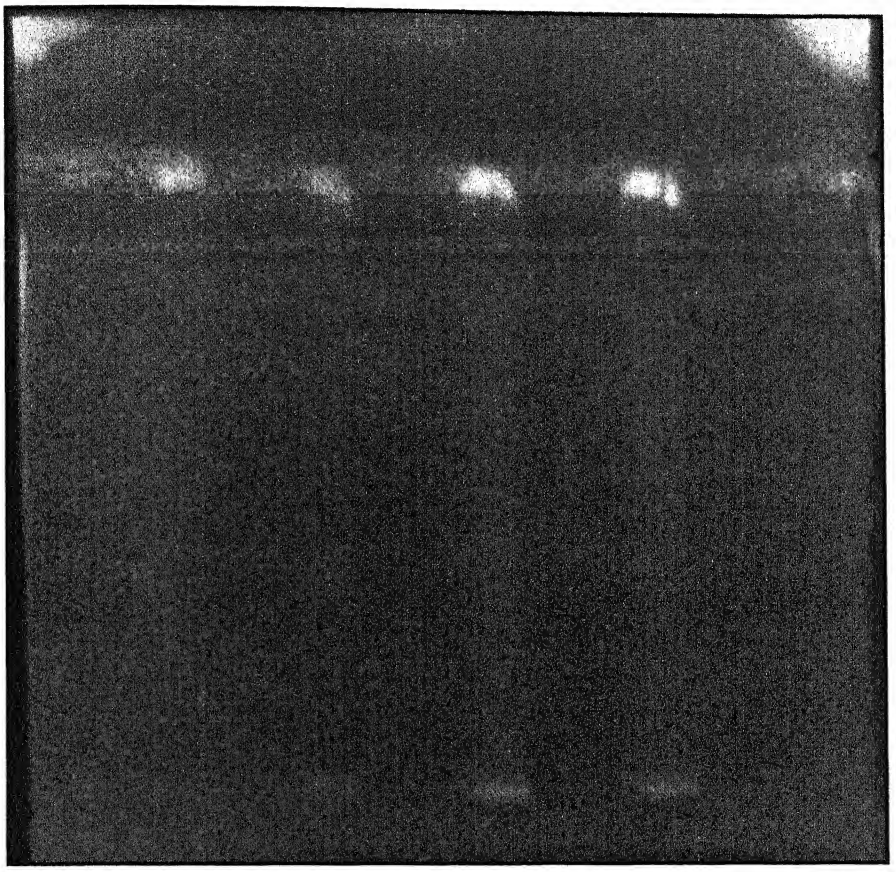


Fig. 31: Chromatogram of Petroleum ether and benzene extracts of seeds of *D.biflorus* observed at 366 nm using solvent system acetone: water (9:1)

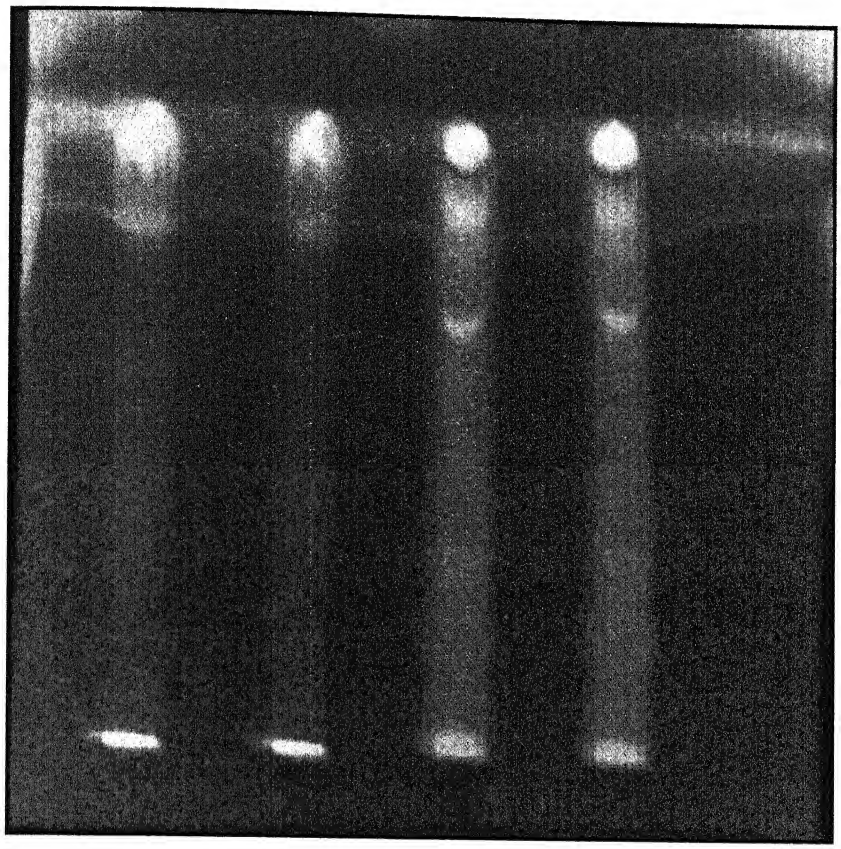


Fig. 32: Chromatogram, of chloroform and ethanol extracts of seeds of *D.biflorus* observed at 366 nm using solvent system acetone: water (9:1)

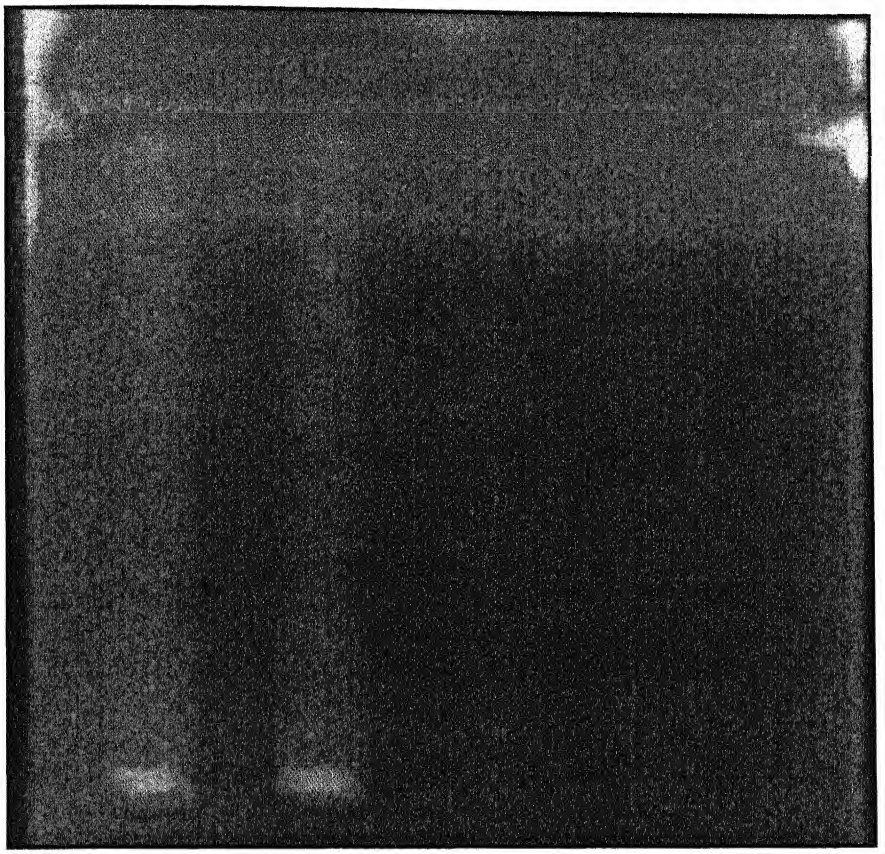
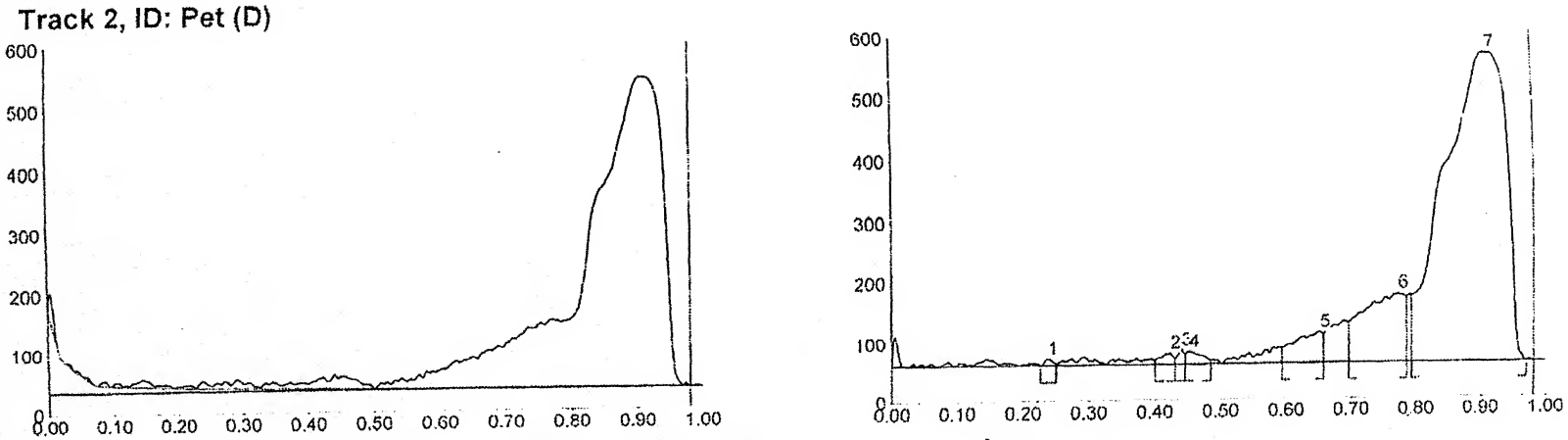
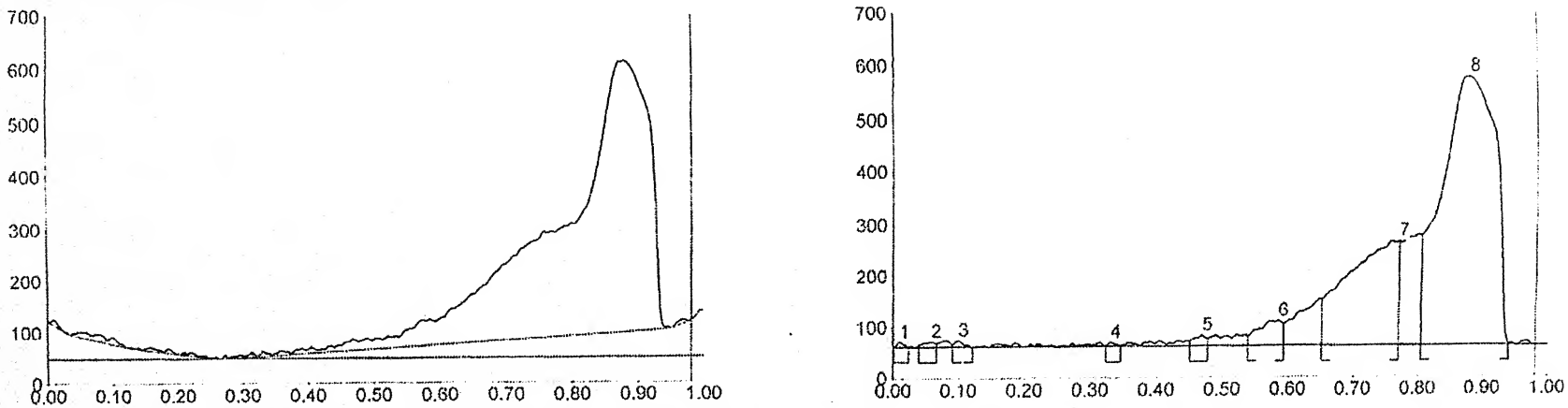


Fig. 33: Chromatogram of aqueous extract of seeds of *D.biflorus* along with authentic sugars observed at 366 nm using solvent system acetone: water (9:1)



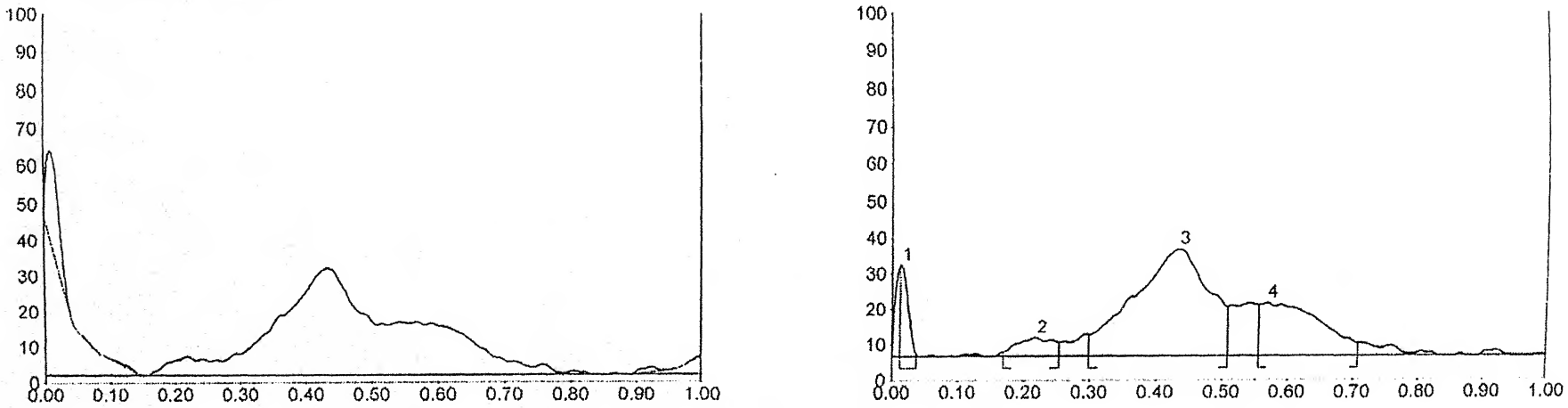
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.23	0.9	0.24	12.1	1.61	0.25	4.3	122.0	0.25	unknown *
2	0.40	7.9	0.42	19.7	2.63	0.43	11.4	313.1	0.65	unknown *
3	0.43	11.4	0.44	24.4	3.25	0.45	17.7	217.8	0.45	unknown *
4	0.45	17.7	0.45	21.7	2.90	0.49	7.0	452.4	0.94	unknown *
5	0.59	27.0	0.65	52.4	6.98	0.66	49.0	1881.1	3.91	unknown *
6	0.70	68.1	0.77	114.0	15.21	0.79	110.0	6212.3	12.92	unknown *
7	0.79	112.4	0.91	505.4	67.42	0.97	0.4	38868.6	80.86	unknown *

Fig. 34: HPTLC Chromatogram of petroleum ether extract of seeds of *D.biflorus* scanned at 254 nm using solvent system n-butanol: acetic acid: water (8:2:2)



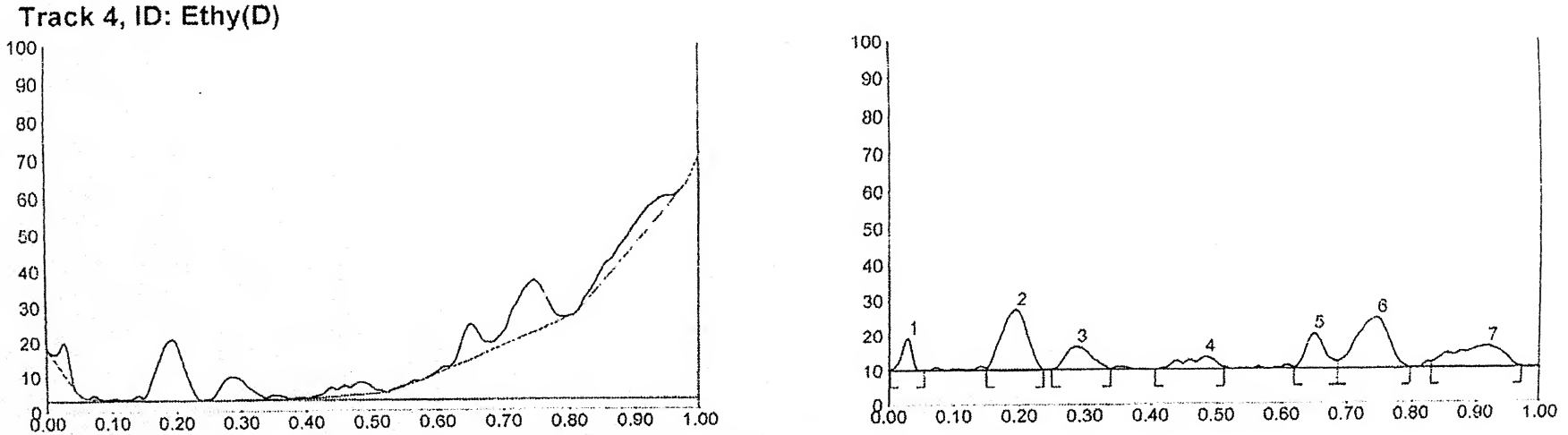
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	1.3	0.01	12.0	1.26	0.02	0.5	101.4	0.18	unknown *
2	0.04	4.8	0.06	13.1	1.37	0.07	9.3	200.3	0.35	unknown *
3	0.09	5.4	0.10	15.1	1.59	0.12	0.1	169.2	0.30	unknown *
4	0.32	0.0	0.33	10.4	1.09	0.35	2.9	94.5	0.17	unknown *
5	0.45	12.4	0.47	24.8	2.60	0.48	18.3	388.5	0.68	unknown *
6	0.54	23.7	0.59	56.0	5.88	0.59	50.1	1726.7	3.02	unknown *
7	0.65	104.8	0.77	233.0	24.45	0.77	232.7	14947.8	26.17	unknown *
8	0.81	246.1	0.88	588.4	61.76	0.94	3.4	39481.0	69.13	unknown *

Fig. 36: HPTLC Chromatogram of authentic amino acids scanned at 254 nm using solvent system n-butanol: acetic acid: water (8:2:2)



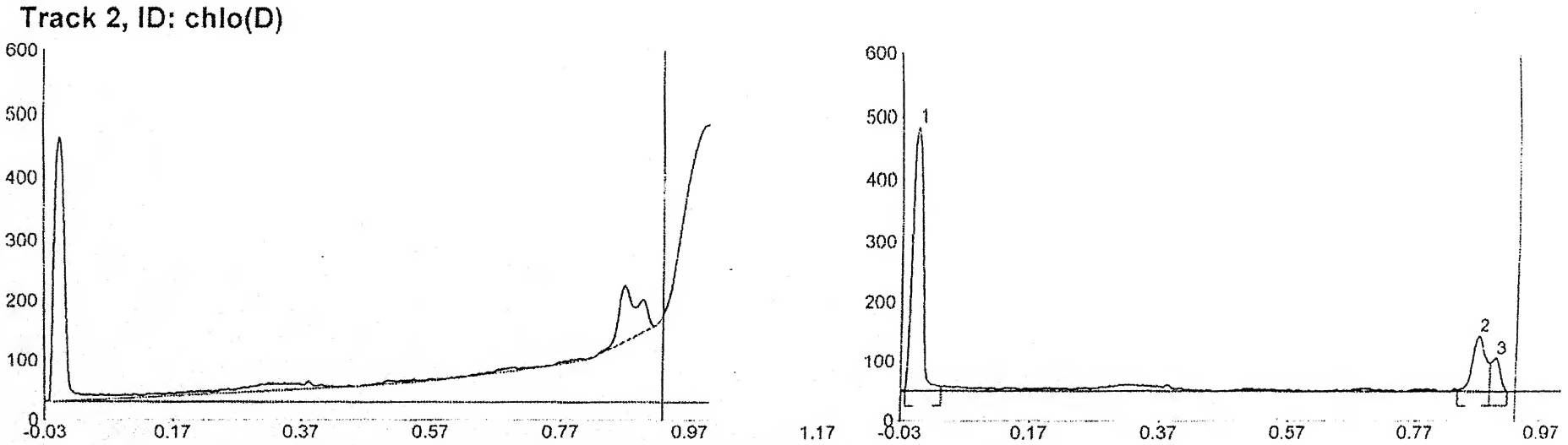
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	76.8	0.01	77.0	33.79	0.04	1.2	456.8	6.05	unknown *
2	0.17	3.8	0.22	15.8	6.93	0.26	11.6	410.0	5.43	unknown *
3	0.30	18.1	0.44	90.0	39.51	0.51	41.9	4725.4	62.63	unknown *
4	0.56	43.4	0.57	45.1	19.78	0.71	10.6	1952.4	25.88	unknown *

Fig. 37: HPTLC Chromatogram of chloroform extract of seeds of *D. biflorus* scanned at 254 nm, using solvent system n-butanol: acetic acid: water (8:2:2)



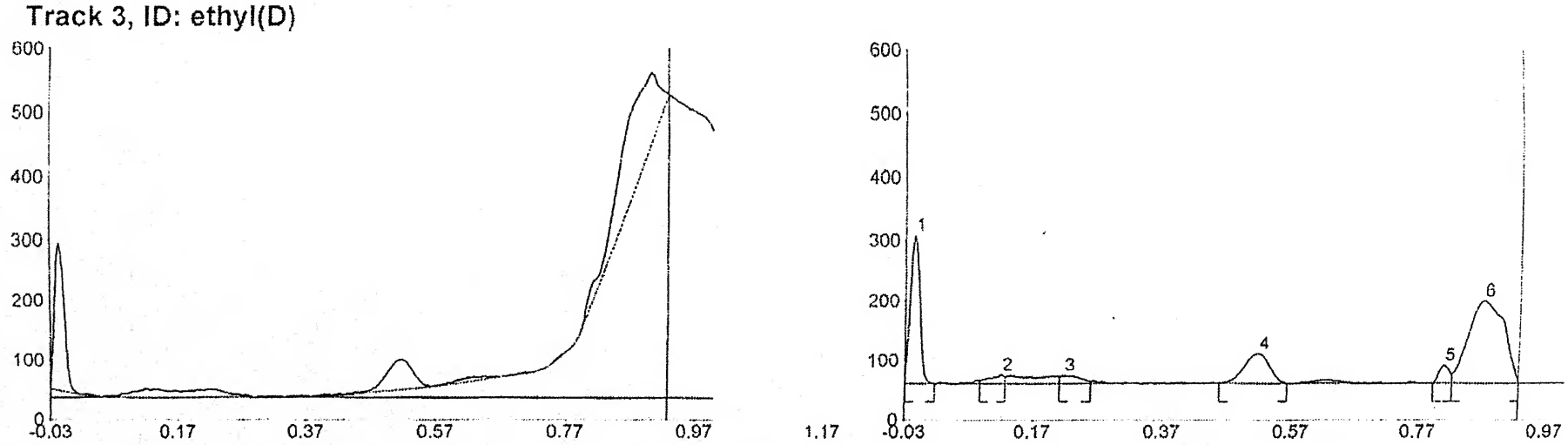
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	0.4	0.03	28.1	13.80	0.06	0.0	205.6	5.24	unknown *
2	0.15	2.2	0.20	52.4	25.72	0.24	0.4	966.6	24.65	unknown *
3	0.25	1.0	0.29	20.1	9.86	0.34	1.2	383.3	9.77	unknown *
4	0.41	0.0	0.48	10.7	5.25	0.51	2.3	254.0	6.48	unknown *
5	0.62	1.7	0.65	30.3	14.88	0.69	6.2	417.4	10.64	unknown *
6	0.69	6.2	0.75	43.8	21.48	0.80	0.8	1019.0	25.98	unknown *
7	0.83	5.2	0.92	18.4	9.02	0.97	0.7	676.1	17.24	unknown *

Fig. 38: HPTLC Chromatogram of ethanol extract of seeds of *D. biflorus* scanned at 254 nm, using solvent system n-butanol: acetic acid: water (8:2:2)



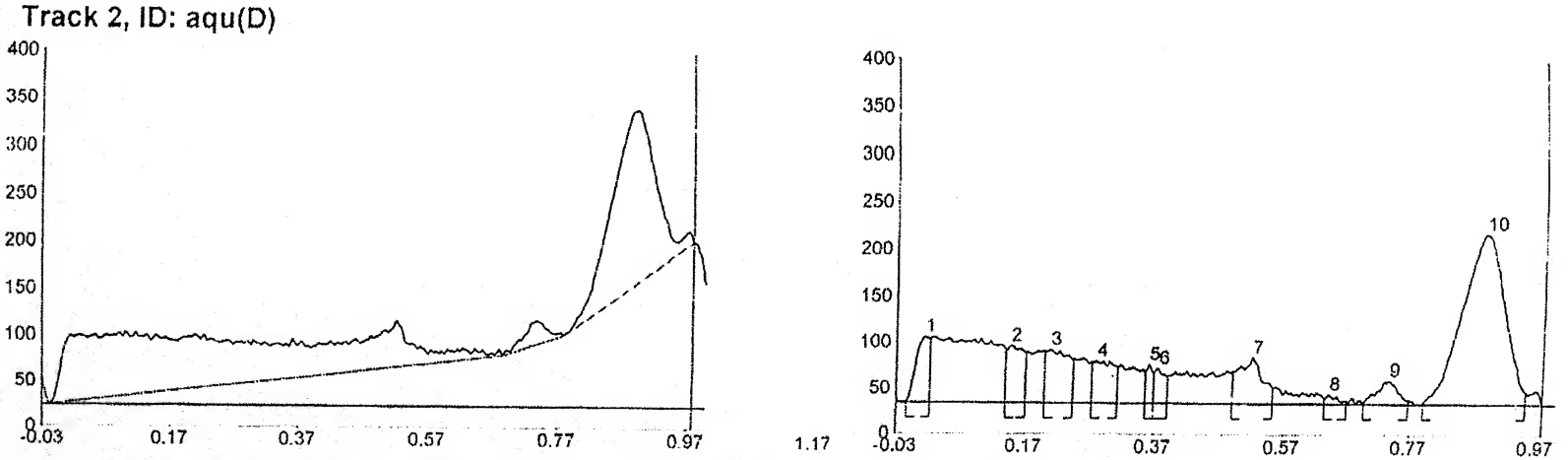
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.4	-0.00	433.8	74.51	0.03	8.4	5609.4	71.37	unknown *
2	0.84	2.6	0.87	93.5	16.06	0.89	47.8	1572.9	20.01	unknown *
3	0.89	47.8	0.90	54.9	9.43	0.92	0.5	677.6	8.62	unknown *

Fig. 39: HPTLC Chromatogram of chloroform extract of seeds of *D. biflorus* scanned at 254 nm using solvent system 96% ethanol: water (7:3)



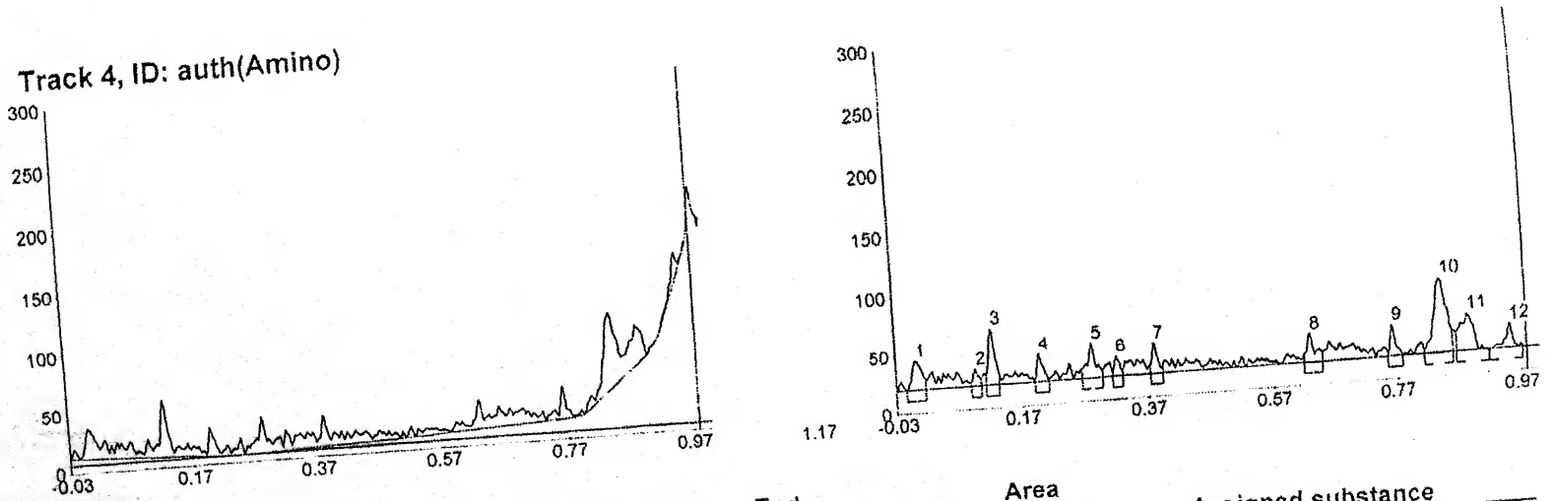
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	16.3	-0.01	247.0	49.88	0.02	0.0	2892.6	24.72	unknown *
2	0.09	5.9	0.12	14.6	2.94	0.13	12.2	289.9	2.48	unknown *
3	0.21	11.9	0.22	13.3	2.69	0.26	4.5	345.8	2.96	unknown *
4	0.46	1.1	0.52	50.8	10.26	0.56	0.2	1598.4	13.66	unknown *
5	0.79	0.2	0.81	30.7	6.19	0.82	16.1	387.5	3.31	unknown *
6	0.82	16.1	0.87	138.9	28.04	0.93	0.0	6187.2	52.88	unknown *

Fig. 40: HPTLC Chromatogram of ethanol extract of seeds of *D. biflorus* scanned at 254 nm using solvent system 96% ethanol: water (7:3)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	2.2	0.01	124.9	12.16	0.02	122.3	2112.7	6.58	unknown *
2	0.14	99.9	0.15	108.7	10.59	0.17	96.7	2251.5	7.02	unknown *
3	0.20	99.1	0.21	101.2	9.86	0.25	82.6	2903.0	9.05	unknown *
4	0.28	78.8	0.28	79.5	7.74	0.31	69.1	2114.8	6.59	unknown *
5	0.36	59.9	0.36	73.4	7.15	0.37	60.3	653.5	2.04	unknown *
6	0.37	60.3	0.38	66.6	6.49	0.39	52.7	950.0	2.96	unknown *
7	0.49	62.5	0.52	87.4	8.51	0.56	30.1	2675.1	8.34	unknown *
8	0.64	10.0	0.64	18.5	1.80	0.67	6.5	263.2	0.82	unknown *
9	0.70	3.5	0.74	45.0	4.39	0.77	7.8	1201.1	3.74	unknown *
10	0.79	3.0	0.89	321.5	31.32	0.95	24.1	16962.1	52.86	unknown *

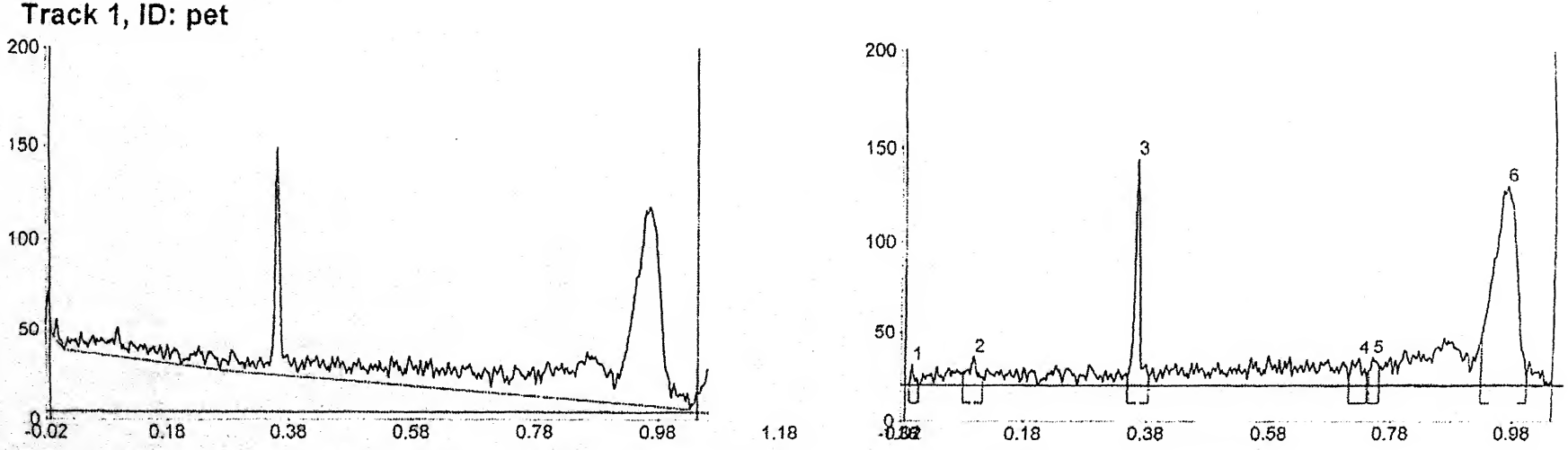
Fig. 41: HPTLC Chromatogram of aqueous extract of seeds of *D. biflorus* scanned at 366 nm using solvent system 96% ethanol: water (7:3)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	1.0	0.00	25.1	7.02	0.02	9.5	313.3	8.10	unknown *
2	0.09	3.1	0.10	15.0	4.19	0.11	6.4	105.8	2.74	unknown *
3	0.11	8.7	0.13	48.8	13.64	0.14	11.1	402.1	10.40	unknown *
4	0.19	1.1	0.20	24.0	6.71	0.21	1.9	173.8	4.49	unknown *
5	0.27	8.5	0.28	30.3	8.47	0.30	5.4	314.5	8.13	unknown *
6	0.32	2.5	0.32	17.3	4.83	0.33	4.9	116.2	3.00	unknown *
7	0.38	7.1	0.38	26.4	7.37	0.40	6.7	187.7	4.85	unknown *
8	0.62	7.5	0.63	25.0	6.99	0.65	8.2	256.5	6.63	unknown *
9	0.76	2.0	0.76	27.7	7.73	0.78	1.2	184.3	4.77	unknown *
10	0.82	2.7	0.85	63.7	17.80	0.86	18.6	1055.6	27.30	unknown *
11	0.87	17.1	0.89	33.0	9.21	0.92	0.4	560.9	14.50	unknown *
12	0.92	0.0	0.95	21.6	6.04	0.97	0.3	196.4	5.08	unknown *

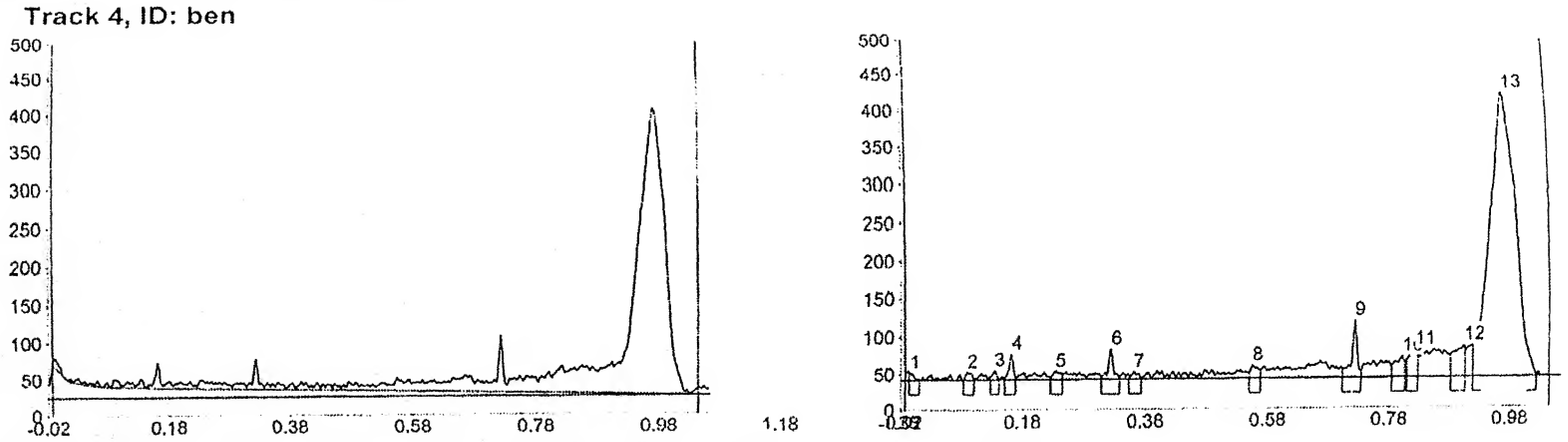
Fig. 42: HPTLC Chromatogram of authentic amino acids scanned at 366 nm, using solvent system

96% ethanol: water (7:3)



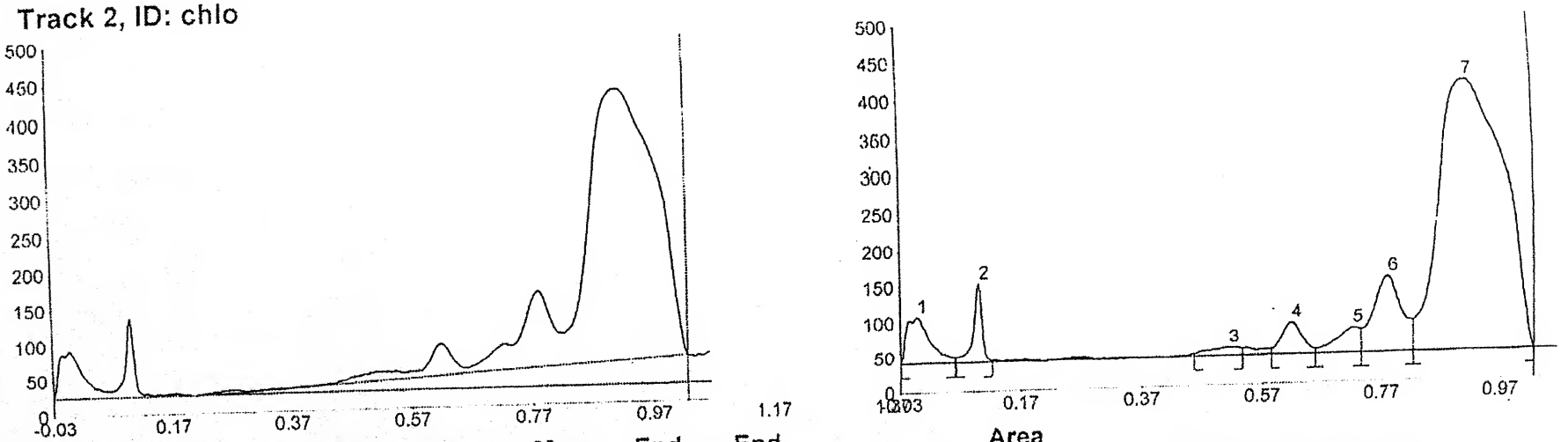
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	1.4	-0.01	11.9	4.06	0.00	0.0	59.3	1.07	unknown *
2	0.07	7.3	0.09	16.2	5.51	0.10	4.6	214.7	3.86	unknown *
3	0.33	4.5	0.34	124.1	42.28	0.36	5.9	976.0	17.57	unknown *
4	0.67	3.0	0.68	15.5	5.29	0.69	5.2	242.3	4.36	unknown *
5	0.70	8.7	0.70	16.0	5.44	0.71	10.9	177.0	3.19	unknown *
6	0.87	29.0	0.91	109.9	37.42	0.94	5.4	3885.9	69.95	unknown *

Fig. 43: HPTLC Chromatogram of petroleum ether extract of seeds of *D. biflorus* scanned at 366 nm using solvent system chloroform : methanol: (6:4)



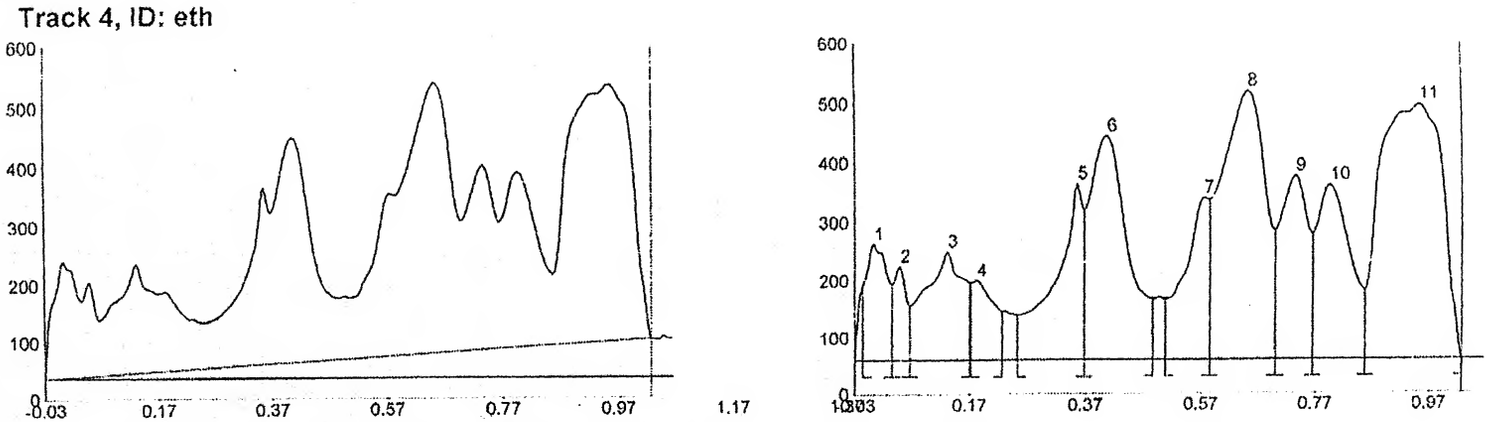
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	14.3	-0.01	14.3	1.67	0.00	2.0	94.5	0.44	unknown *
2	0.07	0.0	0.08	12.8	1.50	0.09	3.2	114.3	0.54	unknown *
3	0.12	5.5	0.12	15.2	1.78	0.13	0.2	90.7	0.42	unknown *
4	0.14	3.4	0.15	41.9	4.90	0.15	4.3	274.5	1.28	unknown *
5	0.21	3.3	0.22	13.6	1.59	0.23	9.8	157.1	0.74	unknown *
6	0.29	2.8	0.30	48.7	5.71	0.31	3.2	363.9	1.70	unknown *
7	0.33	8.0	0.34	11.5	1.35	0.35	0.9	120.2	0.56	unknown *
8	0.51	8.6	0.52	20.7	2.43	0.53	13.6	232.9	1.09	unknown *
9	0.66	14.4	0.68	91.0	10.65	0.69	10.7	771.8	3.61	unknown *
10	0.74	16.8	0.75	33.9	3.96	0.76	25.6	466.9	2.19	unknown *
11	0.76	29.5	0.77	44.5	5.21	0.78	32.7	544.0	2.55	unknown *
12	0.83	35.7	0.85	51.1	5.98	0.85	45.0	859.6	4.02	unknown *
13	0.86	49.3	0.92	454.9	53.27	0.96	2.1	17273.3	80.85	unknown *

Fig. 44: HPTLC Chromatogram of benzene extract of seeds of *D. biflorus* scanned at 366 nm using solvent system chloroform: methanol (6:4)



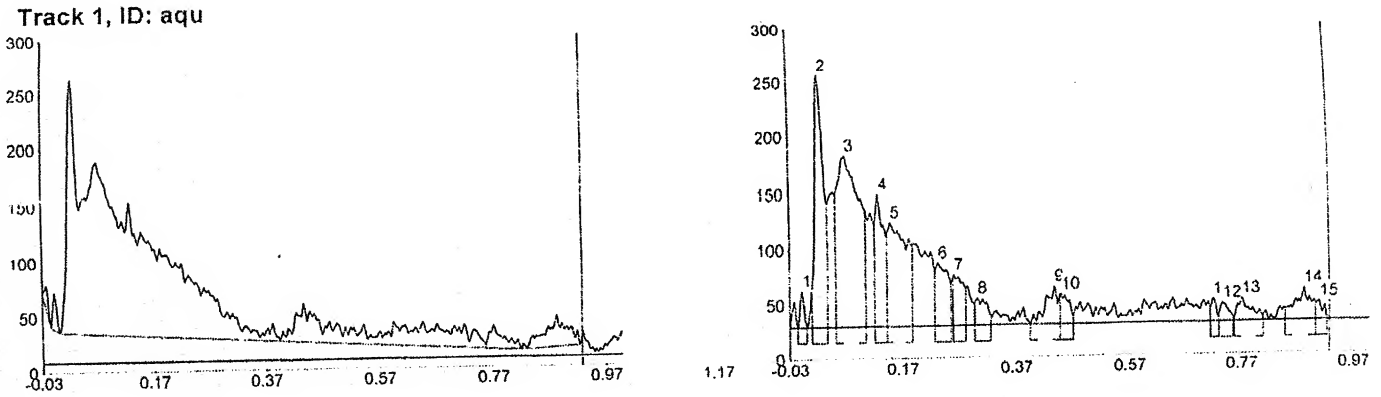
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	2.1	0.00	80.1	8.93	0.06	9.5	2495.3	4.87	unknown *
2	0.06	9.2	0.10	133.1	14.85	0.12	3.4	1558.1	3.04	unknown *
3	0.43	5.1	0.48	15.0	1.67	0.51	12.1	651.5	1.27	unknown *
4	0.55	10.2	0.58	54.6	6.09	0.62	8.5	1549.4	3.03	unknown *
5	0.62	8.5	0.68	44.2	4.93	0.69	41.7	1427.1	2.79	unknown *
6	0.69	41.7	0.73	128.0	14.28	0.77	54.8	4982.2	9.73	unknown *
7	0.77	54.8	0.86	441.7	49.26	0.96	2.2	38540.8	75.27	unknown *

Fig. 45: HPTLC Chromatogram of chloroform extract of seeds of *D. biflorus* scanned at 254 nm using solvent system chloroform: methanol (6:4)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	132.5	0.00	202.5	6.37	0.03	134.1	6089.3	3.82	unknown *
2	0.03	134.1	0.05	164.5	5.18	0.06	97.6	3011.1	1.89	unknown *
3	0.06	97.6	0.12	189.4	5.96	0.16	137.6	10168.2	6.37	unknown *
4	0.16	137.8	0.17	140.3	4.41	0.21	88.7	4454.8	2.79	unknown *
5	0.24	81.2	0.34	304.6	9.58	0.35	262.1	12211.8	7.66	unknown *
6	0.35	262.1	0.38	384.5	12.09	0.46	109.3	20450.8	12.82	unknown *
7	0.48	108.4	0.54	280.1	8.81	0.55	277.4	10065.3	6.31	unknown *
8	0.55	277.4	0.61	459.3	14.45	0.66	225.3	28343.9	17.77	unknown *
9	0.66	225.3	0.69	316.5	9.95	0.72	218.2	12396.6	7.77	unknown *
10	0.72	218.2	0.75	301.1	9.47	0.81	122.4	14424.4	9.04	unknown *
11	0.81	122.4	0.90	436.4	13.73	0.96	2.6	37897.4	23.76	unknown *

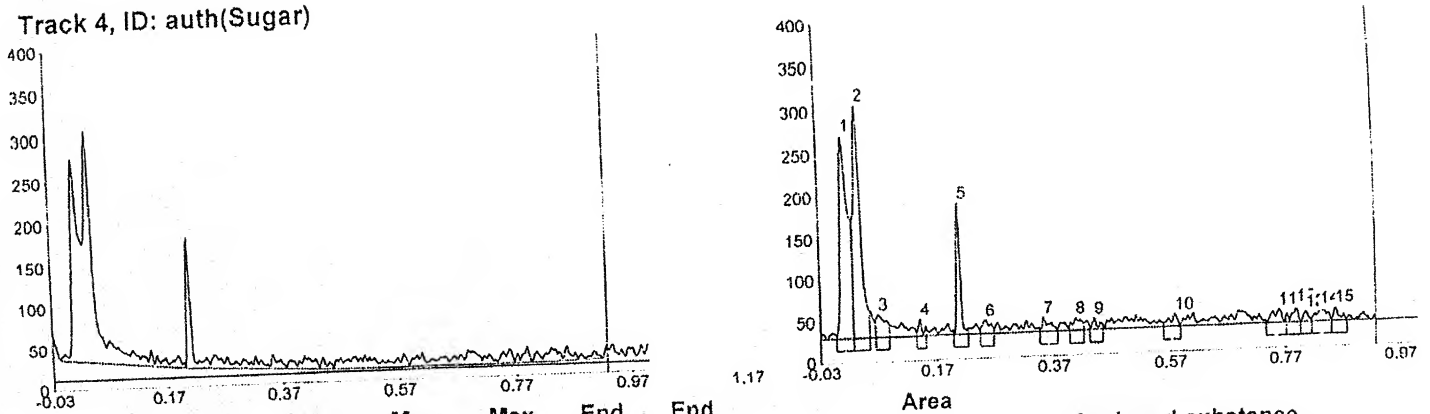
Fig. 46: HPTLC Chromatogram of ethanol extract of seeds of *D. biflorus* scanned at 254 nm, using solvent system chloroform: methanol (6:4)



winCATS Planar Chromatography Manager

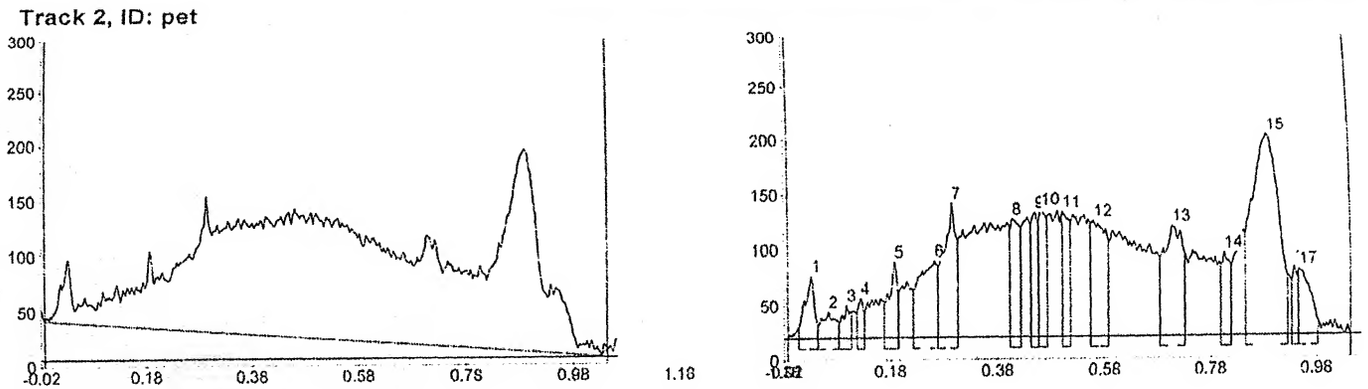
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.0	-0.01	32.8	3.58	0.00	0.1	173.4	1.06	unknown *
2	0.01	33.1	0.03	227.0	24.73	0.04	111.2	2844.4	17.36	unknown *
3	0.05	119.7	0.07	153.1	16.68	0.11	106.7	4813.5	29.38	unknown *
4	0.12	93.4	0.13	119.1	12.98	0.14	81.7	1568.0	9.57	unknown *
5	0.14	81.7	0.15	94.3	10.28	0.19	73.0	2539.0	15.50	unknown *
6	0.23	49.1	0.23	56.8	6.19	0.26	38.3	994.3	6.07	unknown *
7	0.26	44.1	0.26	45.8	4.99	0.28	33.2	642.9	3.92	unknown *
8	0.30	20.1	0.30	24.5	2.67	0.33	8.2	378.6	2.31	unknown *
9	0.40	0.6	0.44	34.2	3.72	0.45	26.8	581.6	3.55	unknown *
10	0.45	26.8	0.45	26.9	2.93	0.47	6.8	335.3	2.05	unknown *
11	0.72	11.9	0.72	20.5	2.23	0.73	5.1	145.2	0.89	unknown *
12	0.73	5.1	0.74	16.7	1.82	0.76	5.4	194.6	1.19	unknown *
13	0.76	2.3	0.77	20.6	2.24	0.81	4.3	375.6	2.29	unknown *
14	0.85	10.5	0.88	28.8	3.14	0.90	14.7	623.5	3.81	unknown *
15	0.90	14.7	0.91	16.8	1.83	0.93	0.7	171.5	1.05	unknown *

Fig. 47: HPTLC Chromatogram of aqueous extract of seeds of *D. biflorus* scanned at 366 nm using solvent system chloroform : methanol (6:4)



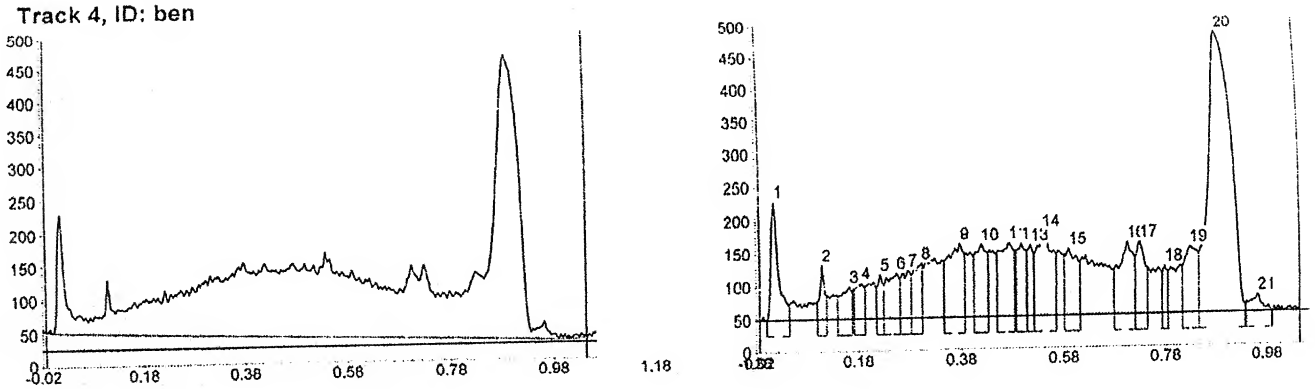
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.00	3.1	0.01	241.3	27.40	0.03	142.3	2808.1	32.69	unknown *
2	0.03	142.3	0.04	275.5	31.29	0.06	31.5	2816.8	32.80	unknown *
3	0.07	22.3	0.07	27.8	3.16	0.09	16.1	366.4	4.27	unknown *
4	0.14	4.2	0.14	19.7	2.24	0.15	12.5	115.4	1.34	unknown *
5	0.20	6.5	0.21	157.9	17.93	0.23	8.0	815.0	9.49	unknown *
6	0.25	3.9	0.25	16.1	1.83	0.27	5.7	183.3	2.13	unknown *
7	0.35	2.7	0.35	16.6	1.88	0.38	1.8	167.5	1.95	unknown *
8	0.40	7.5	0.41	15.2	1.72	0.42	8.5	187.6	2.18	unknown *
9	0.43	1.5	0.44	13.8	1.56	0.46	6.8	106.9	1.24	unknown *
10	0.56	5.6	0.58	15.5	1.77	0.59	0.6	163.6	1.90	unknown *
11	0.74	3.4	0.76	16.7	1.90	0.77	10.6	224.8	2.62	unknown *
12	0.77	10.6	0.79	19.3	2.19	0.80	1.6	169.1	1.97	unknown *
13	0.80	1.6	0.81	12.6	1.43	0.82	3.0	88.6	1.03	unknown *
14	0.82	3.0	0.83	15.9	1.80	0.85	1.6	237.5	2.77	unknown *
15	0.85	3.0	0.86	16.7	1.90	0.88	1.4	138.3	1.61	unknown *

Fig. 48: HPTLC Chromatogram of authentic sugars scanned at 366 nm using solvent system chloroform: methanol (6:4)



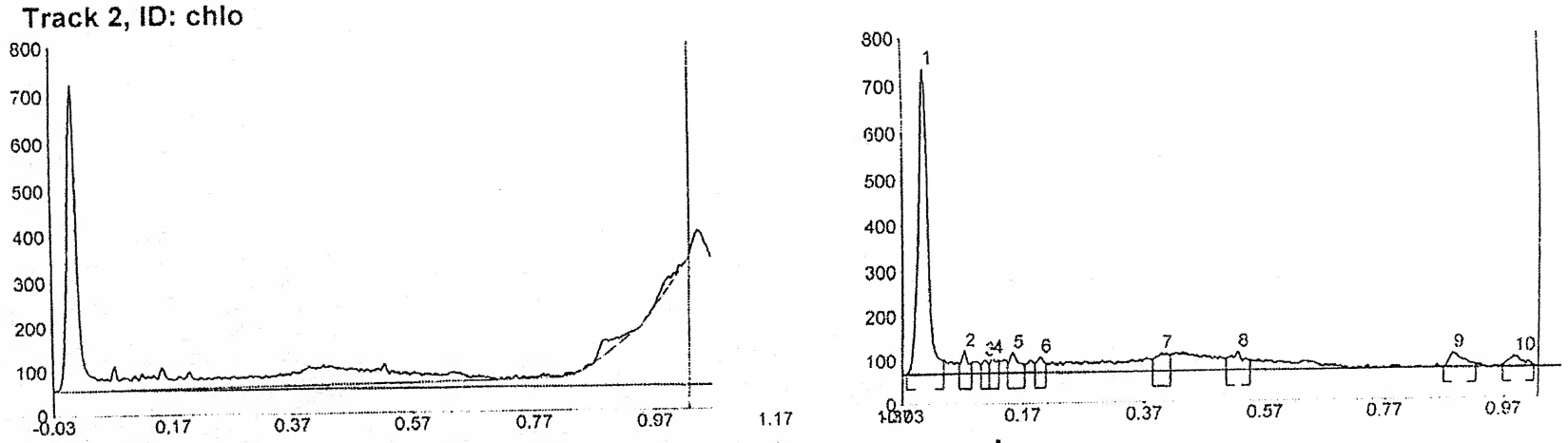
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	8.9	0.03	56.7	3.94	0.04	13.3	921.3	2.94	unknown *
2	0.04	11.3	0.06	24.5	1.71	0.07	13.8	545.7	1.74	unknown *
3	0.07	13.8	0.09	30.5	2.12	0.10	25.3	434.5	1.38	unknown *
4	0.11	25.9	0.11	36.8	2.56	0.12	22.5	360.9	1.15	unknown *
5	0.15	34.4	0.17	68.9	4.79	0.18	41.5	1015.1	3.23	unknown *
6	0.21	44.6	0.24	69.4	4.83	0.25	65.8	2136.2	6.81	unknown *
7	0.25	64.8	0.27	122.1	8.49	0.28	87.5	2636.8	8.40	unknown *
8	0.38	95.7	0.38	106.5	7.41	0.40	99.2	1737.8	5.54	unknown *
9	0.41	100.7	0.42	112.3	7.81	0.43	101.4	1287.2	4.10	unknown *
10	0.43	101.4	0.43	115.6	8.04	0.44	107.2	1536.3	4.89	unknown *
11	0.47	112.2	0.47	112.7	7.84	0.48	102.4	1405.8	4.48	unknown *
12	0.52	102.8	0.53	104.1	7.24	0.55	87.3	2647.0	8.43	unknown *
13	0.64	71.0	0.67	99.0	6.89	0.69	75.6	3221.8	10.26	unknown *
14	0.75	65.0	0.76	74.8	5.20	0.77	63.1	1153.6	3.68	unknown *
15	0.80	92.0	0.84	182.2	12.67	0.87	54.5	8471.9	26.99	unknown *
16	0.88	50.0	0.88	61.9	4.30	0.89	58.0	617.4	1.97	unknown *
17	0.89	58.0	0.89	59.6	4.15	0.93	14.4	1258.6	4.01	unknown *

Fig. 49: HPTLC Chromatogram of petroleum ether extract of seeds of *D. biflorus* scanned at 366 nm using solvent system acetone: water (9:1)



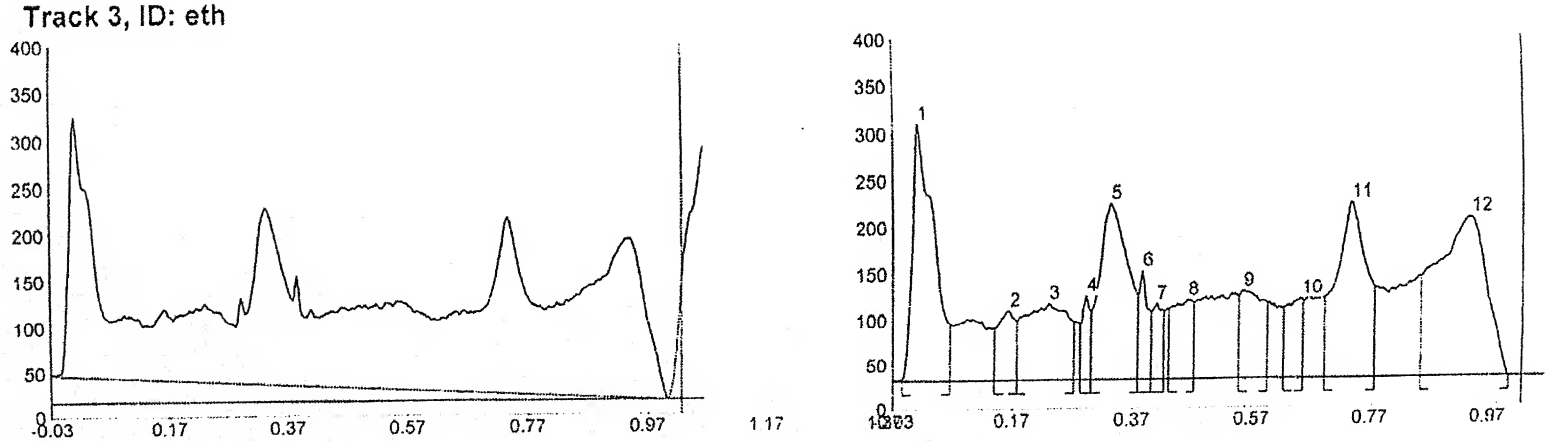
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.00	0.1	0.01	178.5	7.89	0.04	22.9	2356.6	4.53	unknown *
2	0.09	27.7	0.10	81.2	3.59	0.11	36.2	712.0	1.37	unknown *
3	0.13	34.7	0.15	48.3	2.13	0.15	40.8	930.0	1.79	unknown *
4	0.16	46.2	0.17	52.9	2.34	0.18	50.7	890.9	1.71	unknown *
5	0.20	47.9	0.21	65.6	2.90	0.21	50.4	660.9	1.27	unknown *
6	0.21	50.4	0.24	67.4	2.98	0.24	60.0	1560.6	3.00	unknown *
7	0.24	60.0	0.26	71.5	3.16	0.26	65.9	1174.5	2.26	unknown *
8	0.26	65.9	0.28	82.0	3.62	0.28	81.3	1340.0	2.57	unknown *
9	0.32	84.2	0.35	110.1	4.87	0.36	95.1	3180.4	6.11	unknown *
10	0.38	93.5	0.39	108.3	4.79	0.40	97.0	2295.9	4.41	unknown *
11	0.42	96.7	0.44	110.2	4.87	0.45	99.0	2846.5	5.47	unknown *
12	0.46	98.0	0.47	109.3	4.83	0.47	99.0	1630.4	3.13	unknown *
13	0.48	99.2	0.48	107.1	4.73	0.49	95.1	1011.5	1.94	unknown *
14	0.49	93.5	0.50	127.4	5.63	0.53	91.7	3667.7	7.05	unknown *
15	0.54	92.1	0.55	99.4	4.39	0.57	80.0	2225.9	4.28	unknown *
16	0.64	66.4	0.66	107.9	4.77	0.68	86.2	2881.9	5.54	unknown *
17	0.68	86.2	0.69	109.0	4.82	0.70	68.6	1799.2	3.46	unknown *
18	0.73	59.3	0.73	70.9	3.13	0.74	62.7	650.6	1.25	unknown *
19	0.76	69.2	0.78	99.5	4.40	0.80	89.8	2418.1	4.65	unknown *
20	0.80	89.8	0.84	430.6	19.03	0.88	9.6	17302.9	33.24	unknown *
21	0.88	15.1	0.90	25.5	1.13	0.93	0.4	519.5	1.00	unknown *

Fig 50: HPTLC Chromatogram of benzene extract of seeds of *D. biflorus* scanned at 366 nm using solvent system acetone: water (9:1)



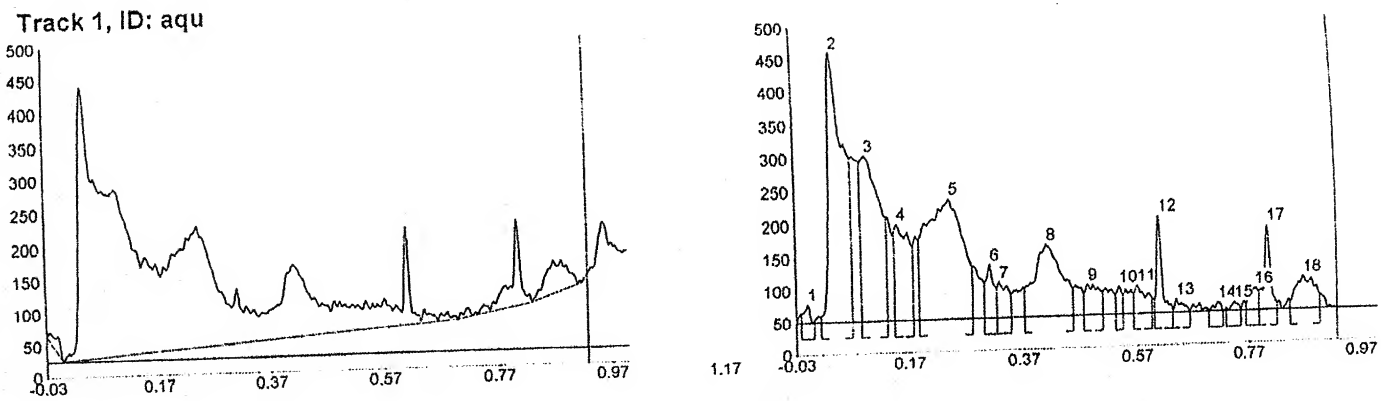
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	2.2	0.01	665.8	65.77	0.04	32.0	8485.2	63.83	unknown *
2	0.06	23.1	0.07	52.4	5.17	0.08	25.5	483.4	3.64	unknown *
3	0.10	21.1	0.10	31.4	3.10	0.11	21.4	260.5	1.96	unknown *
4	0.11	21.4	0.11	35.9	3.55	0.12	29.9	351.2	2.64	unknown *
5	0.14	23.9	0.15	46.9	4.63	0.16	21.0	604.2	4.55	unknown *
6	0.18	22.9	0.19	36.8	3.64	0.20	21.3	363.9	2.74	unknown *
7	0.36	28.6	0.38	41.3	4.08	0.39	39.1	768.8	5.78	unknown *
8	0.48	28.6	0.49	42.6	4.21	0.51	23.0	827.0	6.22	unknown *
9	0.82	1.0	0.83	34.5	3.41	0.87	7.6	670.2	5.04	unknown *
10	0.91	5.1	0.93	24.8	2.45	0.96	1.8	479.3	3.61	unknown *

Fig. 51: HPTLC Chromatogram of Chloroform extract of seeds of *D. biflorus* scanned at 366 nm using solvent system acetone: water (9:1)



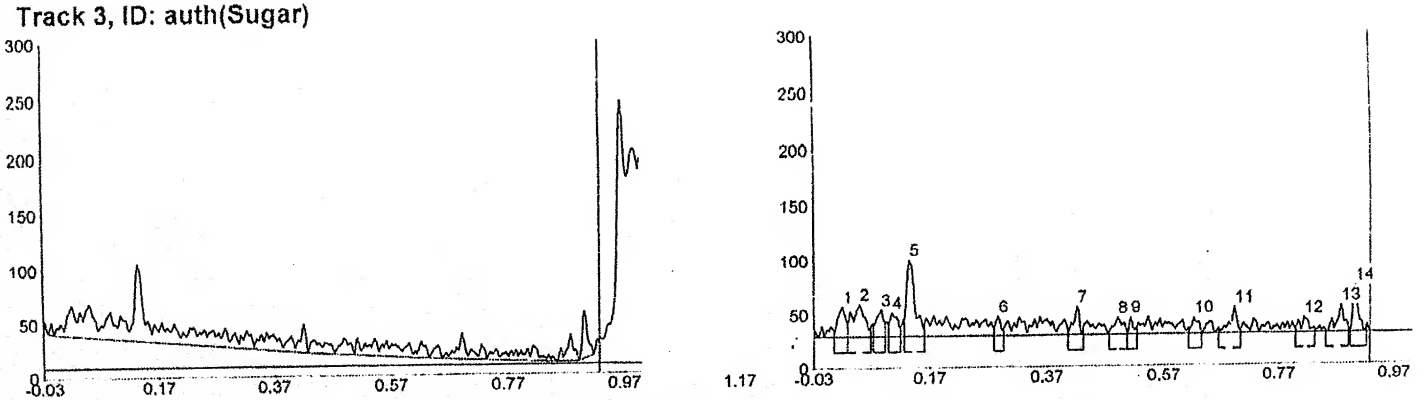
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	2.4	0.01	348.2	17.74	0.06	80.6	9932.3	14.86	unknown *
2	0.13	74.4	0.16	96.9	4.94	0.17	82.8	2338.1	3.50	unknown *
3	0.17	82.8	0.22	106.4	5.42	0.26	81.7	6258.6	9.37	unknown *
4	0.27	78.5	0.28	116.5	5.94	0.28	96.1	1282.7	1.92	unknown *
5	0.28	97.2	0.32	240.1	12.24	0.36	116.1	9318.2	13.94	unknown *
6	0.36	116.1	0.36	149.6	7.62	0.38	94.7	1865.9	2.79	unknown *
7	0.38	94.7	0.39	105.1	5.35	0.40	95.6	1476.5	2.21	unknown *
8	0.41	98.7	0.44	110.1	5.61	0.45	106.8	3143.8	4.70	unknown *
9	0.52	114.5	0.52	121.1	6.17	0.56	106.1	3894.8	5.83	unknown *
10	0.59	99.2	0.61	111.1	5.66	0.62	108.3	2412.0	3.61	unknown *
11	0.65	112.8	0.69	239.0	12.18	0.73	124.9	9825.9	14.70	unknown *
12	0.80	139.3	0.88	218.4	11.13	0.94	0.2	15072.7	22.56	unknown *

Fig. 52: HPTLC Chromatogram of ethanol extract of seeds of *D. biflorus* scanned at 366 nm using solvent system acetone: water (9:1)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	15.2	-0.01	28.3	1.56	0.00	3.8	240.9	0.58	unknown *
2	0.01	9.9	0.04	410.8	22.58	0.07	250.0	8958.3	21.65	unknown *
3	0.09	246.0	0.10	252.3	13.87	0.13	159.9	6851.2	16.55	unknown *
4	0.15	140.4	0.15	147.7	8.12	0.18	115.8	3066.3	7.41	unknown *
5	0.19	124.6	0.24	184.3	10.13	0.28	81.8	9561.7	23.10	unknown *
6	0.30	55.8	0.31	83.2	4.58	0.33	45.9	980.3	2.37	unknown *
7	0.33	45.9	0.33	55.1	3.03	0.35	37.5	833.4	2.01	unknown *
8	0.37	45.8	0.41	111.8	6.14	0.46	43.0	4331.9	10.47	unknown *
9	0.48	32.2	0.49	47.7	2.62	0.51	36.5	974.5	2.35	unknown *
10	0.53	31.0	0.54	43.1	2.37	0.55	30.5	360.2	0.87	unknown *
11	0.57	30.9	0.57	43.8	2.41	0.60	24.9	736.2	1.78	unknown *
12	0.60	19.0	0.61	149.0	8.19	0.64	3.6	1088.5	2.63	unknown *
13	0.64	3.6	0.64	20.3	1.11	0.67	0.2	220.2	0.53	unknown *
14	0.70	0.9	0.72	13.4	0.74	0.72	0.8	123.9	0.30	unknown *
15	0.73	5.0	0.75	13.3	0.73	0.76	1.5	128.3	0.31	unknown *
16	0.77	9.6	0.78	34.3	1.88	0.79	28.1	430.3	1.04	unknown *
17	0.79	28.1	0.81	130.0	7.14	0.82	9.8	1176.1	2.84	unknown *
18	0.85	12.5	0.87	51.1	2.81	0.90	21.4	1323.4	3.20	unknown *

Fig. 53: HPTLC Chromatogram of aqueous extract of seeds of *D. biflorus* scanned at 366 nm using solvent system acetone: water (9:1)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	4.3	0.02	27.4	7.50	0.03	13.3	295.0	7.77	unknown *
2	0.03	13.3	0.05	29.6	8.08	0.07	10.0	517.8	13.63	unknown *
3	0.07	10.7	0.09	24.3	6.65	0.09	12.9	256.4	6.75	unknown *
4	0.10	11.2	0.11	21.5	5.87	0.12	9.2	233.7	6.15	unknown *
5	0.13	15.3	0.14	70.7	19.34	0.16	11.9	812.4	21.39	unknown *
6	0.28	7.9	0.29	17.3	4.73	0.30	6.8	129.4	3.41	unknown *
7	0.41	3.0	0.42	25.8	7.06	0.43	8.2	204.3	5.38	unknown *
8	0.48	2.8	0.49	14.9	4.08	0.51	0.8	184.9	4.87	unknown *
9	0.51	0.8	0.51	15.4	4.20	0.53	6.4	87.1	2.29	unknown *
10	0.61	4.7	0.62	14.6	4.00	0.64	1.3	126.9	3.34	unknown *
11	0.67	4.8	0.69	23.6	6.46	0.70	2.4	225.0	5.93	unknown *
12	0.80	4.9	0.81	13.1	3.59	0.83	2.9	164.8	4.34	unknown *
13	0.85	0.8	0.88	24.5	6.71	0.89	2.2	246.2	6.48	unknown *
14	0.90	0.9	0.90	42.9	11.73	0.92	6.6	313.6	8.26	unknown *

Fig. 54: HPTLC Chromatogram of authentic sugars scanned at 366 nm using solvent system acetone: water (9:1)

Discussion:

The proximate analysis of the seeds of *D.biflorus* was carried out to lay certain standards for the air dried drug. The high value of total ash indicated the presence of inorganic constituents in the seeds. The water-soluble extractive value was also high, indicated the presence of sugars. The qualitative examination of the various solvent extracts of seeds indicated the presence of carbohydrates, sterols, proteins and amionacids, fixed oils and fats and absence of absence of alkaloids, glycosides, saponins, tannis, resins, gums and mucilages. Thin-layer chromatography indicated the presence of eight amino acids viz., alanine, hsitidine, cystine, aspartic acid leucine, glycine, serine and lysine as well as the five various sugars like rhamnose, arabinose, fructose, galactose and glucose by Co-chromatography using authentic sample.

The successive solvent extracts of the seeds of *D.biflorus* with petroleum ether, benzene, chloroform, ethanol and water along with authentic amino acids and sugars were scanned using n-butanol: acetic acid: water (8:2:2), 96% ethanol: water (7:3), chloroform : methanol (6:4) and acetone: water (9:1) as solvent systems by HPTLC as shown in Tables 19 and 20.

(B) MACROSCOPIC CHARACTERS

Fruits contain 5-7 seeds. Seeds are compressed , hard , surface smooth, ellipsoid, flattened; 4 - 6 mm long, 4 - 5 mm wide, 2.5 - 3 mm thick; micropyle prominent; greyish to raddish brown in colour; odourless; taste, somewhat astringent (Fig.. 55).

(C) MICROSCOPIC CHARACTERS

T.S. of Seed and Powder Characteristics

Transverse section of seed shows testa consisting of a single layer of columnar, thin-walled, parenchymatous, palisade like cells covered with a thin cuticle followed by single layer of rectangular to square bearer cells and 3 - 4 layers of thin-walled rectangular parenchymatous cells, more wide at micropyler region ; cotyledon consisting of single layer of upper and lower epidermis covered with a thin cuticle ; epidermal cells thin-walled, rectangular and parenchymatous followed by mesophyll, consisting of angular parenchymatous cells , filled with numerous simple starch grains and protein bodies also present.

Powder is whitish in colour; consisting of broken pieces of testa, parenchymatous cells, aleurone grains and starch[82].

Transverse section of the seed and its powder characteristics were observed under microscope as shown in Fig.s.56 and 57.

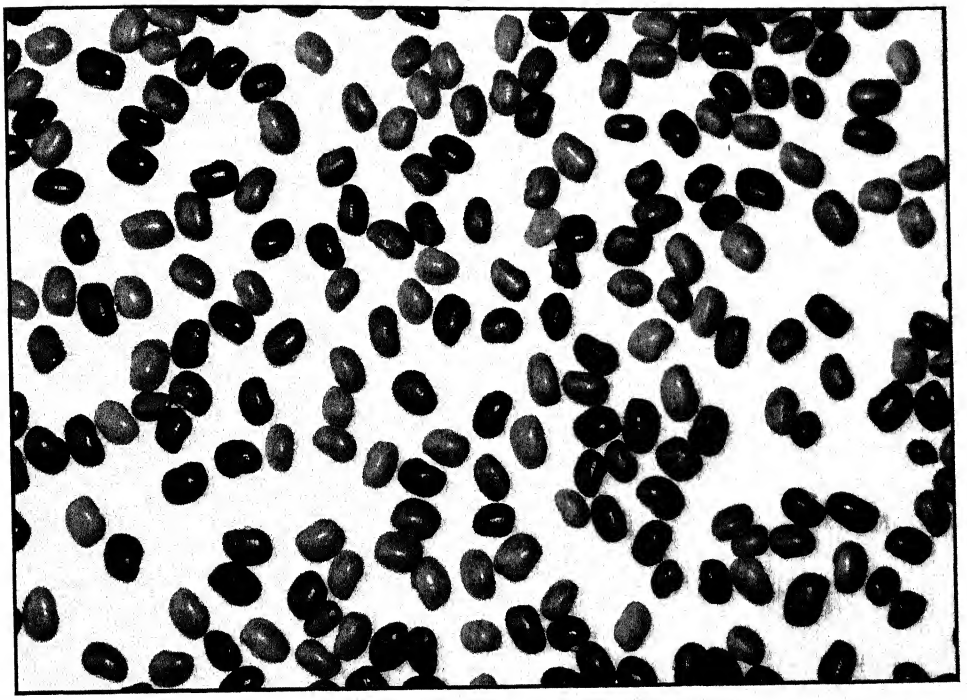


Fig. 55: Seeds of *Dolichos biforus* Linn.

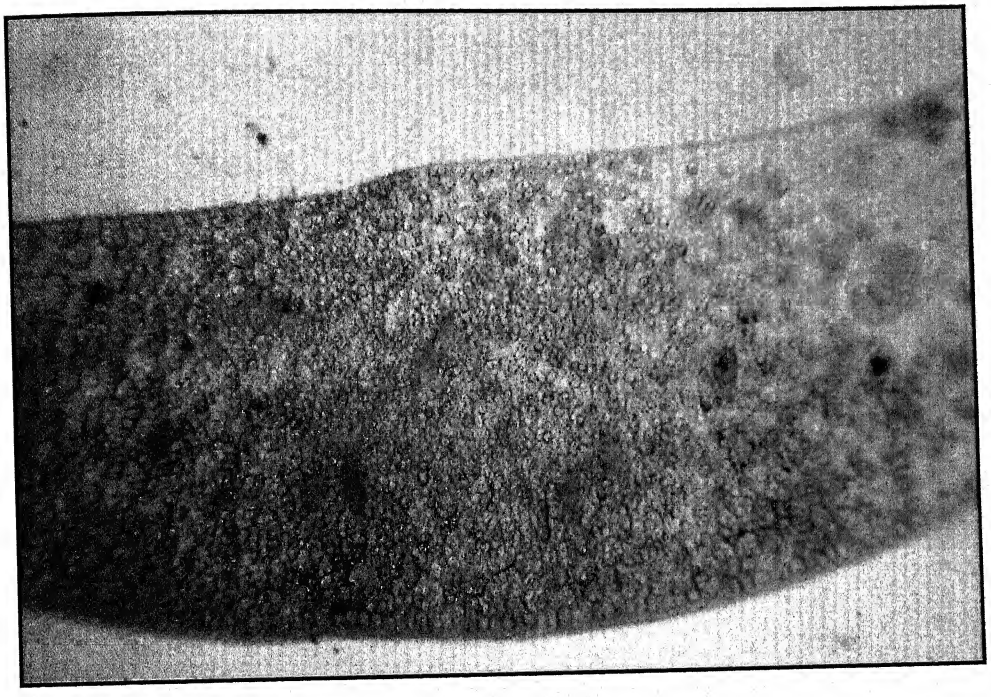


Fig. 56: T.S. of seed of *D. biflorus* Linn. (Celluar)

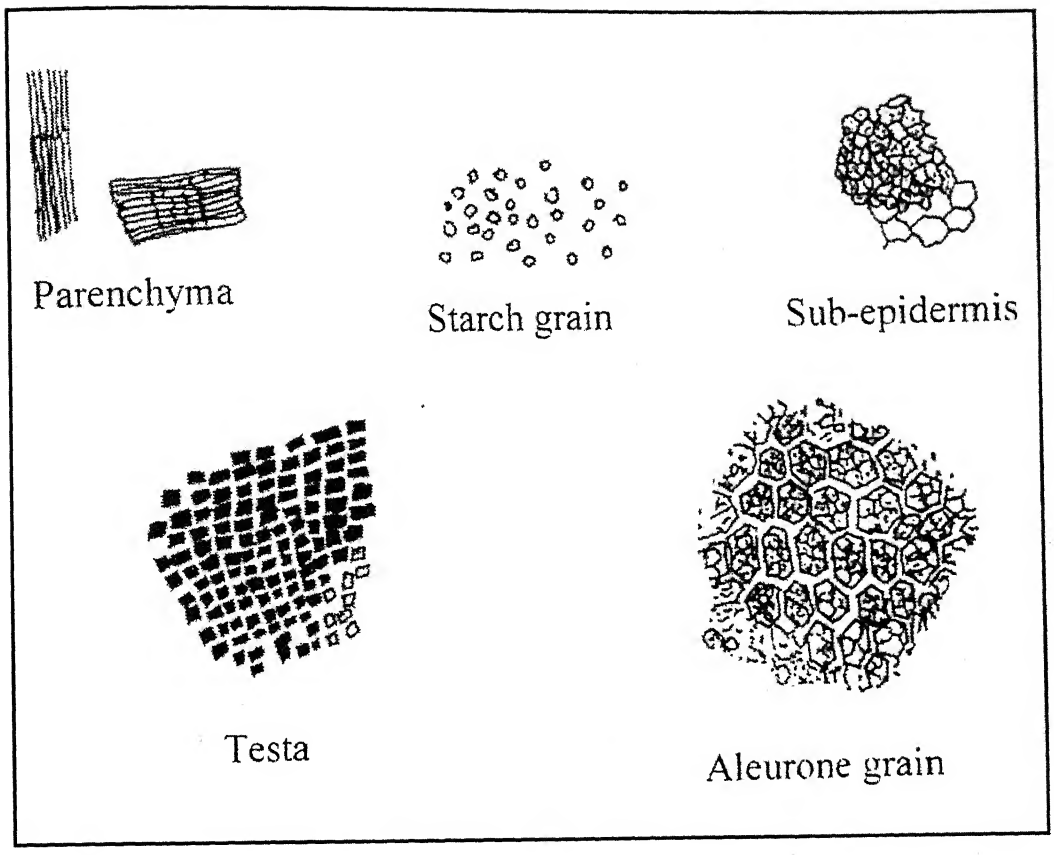


Fig. 57: Powder characteristics of seed of *D. biflorus* Linn.

(D) ANTIMICROBIAL ACTIVITY OF FRUIT OF *TRIBULUS TERRESTRIS* LINN.

EXPERIMENTAL:

The air dried, pulverized fruits of *T. terrestris* were successively extracted with petroleum ether (60-80°) and ethanol (50%) in a Soxhlet extractor. Each extract was concentrated to dryness under reduced pressure. The concentrated ethanol extract and petroleum extract were dissolved in dimethyl sulfoxide (DMSO), an inert solvent which was also used as control and found inert against all the tested microorganisms , i.e., *Staphylococcus aureus* , *Escherichia coli* and *Candida albicans* .

The cultures of *Staphylococcus aureus* , *Escherichia coli* and *Candida albicans* were obtained from Microbial Type Culture Collection , Institute of Microbial Technology , Chandigarh . All these cultures are maintained in Dr. K.N. Modi Institute of Pharmaceutical Education and Research , Modinagar .

The growth medium used for the test microorganisms viz. *Staphylococcus aureus* and *Escherichia coli* was medium No. 1 (Hi-Media) and growth medium used for *Candida albicans* was Sabouraud dextrose agar (Hi-Media). The petri plates were Pre-seeded with 10 ml of growth medium and 4 ml inoculum each of *E. coli* and *S. aureus* and 6.5 ml inoculum of *C. albicans*. The filter paper discs of 6 mm diameter were prepared by soaking in 0.1 ml extract each of ethanol and petroleum ether. The discs were also prepared by soaking known quantity of standard reference antibiotics which were used for comparison of zone of inhibition. These dried discs were placed on the seeded medium already swabbed with test organism.

The inoculated bacterial cultures were incubated at 32 - 35° for 21 h and the fungus culture was incubated at 22 - 25° for 48 h. The antimicrobial activity was assayed by disc diffusion method[83].

The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the disc. The results recorded in terms of the diameters of the zone of inhibition, are presented in Tables 21 and 22.

TABLE 21: ANTIBACTERIAL ACTIVITY OF *TRIBULUS TERRESTRIS* FRUIT EXTRACT

Extract/Antibiotic	**Concentration (per ml)**	**Zone of inhibition (mm)**	
		S. aureus	***E. coli***
Petroleum ether (60-80°) extract	20 µg	8.8	9.2
Ethanol (50%) extract	30 µg	10.5	9.0
Chloramphenicol	30 µg	17.0	15.0

TABLE 22: ANTIFUNGAL ACTIVITY OF *TRIBULUS TERRESTRIS* FRUIT EXTRACT

Extract/Antibiotic	**Concentration (per ml)**	**Zone of inhibition (mm)**
		Candida albicans
Petroleum ether (60-80°) extract	75 µg	17.9
Ethanol (50%) extract	75 µg	16.8
Nystatin	100 units	21.0

DISCUSSION:

It was evident from the results that the ethanol extract and petroleum ether (60-80°) extract of fruit of *Tribulus terrestris* showed positive response against

organisms tested as compared to Chloramphenicol and Nystatin , used as standard drugs.

The chemical nature of the active principle(s) responsible for the antimicrobial activity of the fruit extracts was not established.

(E) ANTIMICROBIAL ACTIVITY OF THE SEEDS OF *CICHORIUM INTYBUS* LINN.

EXPERIMENTAL:

500 g of the seeds powder was successively extracted with petroleum ether (60-80°) and ethanol (95%) in a Soxhlet extractor. Each extract was concentrated to dryness *in vacuo*. Antimicrobial activity of the extracts was determined using paper-disc diffusion method by measuring zone of inhibition. The extracts at a concentration of 30 µg and 60 µg/disc were screened for their antimicrobial activity using *Bacillus subtilis* , *Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa* , *Aspergillus niger* , *Aspergillus flavus* , *Candida albicans* and *Fusarium oxysporum* as test organisms.

The cultures of microorganisms were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. All these cultures are maintained in Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar.

Nutrient agar (Hi-Media) and Sabouraud dextrose agar (Hi-Media) were used as media for bacteria and fungi respectively. Control experiment was carried out under similar condition by using ceftazidime and miconazole as a standard for antibacterial and antifungal activity respectively. The petri dishes were incubated at 37° for 48 h. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the disc.[84]

The results recorded in terms of the diameters of the zone of inhibition, are presented in Table 23.

TABLE 23: ANTIMICROBIAL ACTIVITY OF PETROLEUM ETHER AND ETHANOLIC EXTRACTS OF *CICHORIUM INTYBUS* SEEDS

Microorganisms	Zone of inhibition in mm					
	Petroleum ether (µg/disc)		Ethanol (µg/disc)		Ceftazidime (µg/disc)	Miconazole (µg/disc)
	30	60	30	60	30	10
Bacteria						
Bacillus subtilis	–	+	–	–	+++	NT
Staphylococcus aureus	–	–	+	+	+++	NT
Escherichia coli	–	+	–	–	++	NT
Pseudomonas aeruginosa	–	–	–	+	+++	NT
Fungi						
Aspergillus niger	+	++	++	+++	NT	+++
Aspergillus flavus	++	+++	++	++	NT	+++
Candida albicans	+	++	++	+++	NT	+++
Fusarium oxysporum	++	+++	+	++	NT	+++

Disc diameter = 4 mm

Zone of inhibition (mm): –<4 ; + = 5-10 ; ++ = 11-15 ; +++ = > 16 .

NT = Not Tested

DISCUSSION:

The study revealed that petroleum ether and ethanol extracts exhibited moderate to significant antifungal activity against all the tested fungal organisms at the concentration of 30 µg and 60 µg but none of the extracts was active against the tested bacterial organisms.

(F) ANTIMICROBIAL ACTIVITY OF THE SEEDS OF *DOLICHOS BIFLORUS* LINN.

EXPERIMENTAL:

500 g of the seeds powder was successively extracted with petroleum ether (60-80°) and ethanol (95%) in a soxhlet extractor. The extracts were concentrated to dryness *in vacuo*. The antimicrobial activities of the extracts were evaluated by disc diffusion method as described earlier. Both the extracts at a concentration of 25 µg and 50 µg were screened for their anti microbial activity using *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger* and *Candida albicans* as test organisms.

The cultures of microorganisms were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. All these cultures are maintained in Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar.

The activity of the extracts was compared with the antibacterial and antifungal standards. The Ceftazidime and Ketoconazole were used as standard for antibacterial and antifungal activity respectively. Nutrient agar (Hi-Media) and Sabouraud dextrose agar (Hi-Media) were used as media for bacteria and fungi respectively. The plates were incubated at 37° for 48 h for bacteria and at 26 ± 1° for 72 h for fungi. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the disc. The results recorded in terms of the diameters of the zone of inhibition, are presented in Table 24.

TABLE 24: ANTIMICROBIAL ACTIVITY OF PETROLEUM ETHER AND ETHANOLIC EXTRACTS OF *DOLICHOS BIFLORUS* SEEDS

Microorganisms	Zone of inhibition in mm					
	Petroleum ether (µg/disc)		Ethanol (µg/disc)		Ceftazidime (µg/disc)	Ketoconazole (µg/disc)
	25	50	25	50	25	10
Bacteria						
Bacillus subtilis	–	–	+	+	++	NT
Staphylococcus aureus	–	–	++	++	+++	NT
Escherichia coli	–	+	+	++	+++	NT
Pseudomonas aeruginosa	–	+	–	+	+++	NT
Fungi						
Aspergillus niger	–	+	–	+	NT	+++
Candida albicans	–	–	+	–	NT	+++

Disc diameter = 4 mm

Diameter of zone of inhibition (mm): – = < 4 ; + = 5-10 ; ++ = 10-15 ; +++ = > 16.

NT = Not Tested

DISCUSSION

The study revealed that the ethanol extract exhibited significant antibacterial activity against all the tested bacterial organisms at the concentration of 25 µg and 50 µg. The petroleum ether extract at the concentration of 50 µg showed a slight antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* but none of the extracts was found active against the tested fungal organisms.

CHAPTER - 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

STANDARDIZATION

Small caltrops, *Tribulus terrestris* Linn. (Fig..1), belonging to the family Zygophyllaceae is commonly known as *'Chotagokhru'*, has been described to be of great medicinal value. It is a reputed drug in Ayurvedic system.

The phytochemical study of the fruits of *Tribulus terrestris* was carried out to lay certain standards for the air dried drug. The high value of total ash 12.79% indicated the presence of considerable amount of inorganic constituents in the fruits. The ethanol-soluble extractive and water-soluble extractive values , 1.862% and 16.8% respectively were also rather high , indicated the presence of sugars and resins etc. The qualitative chemical examination of the petroleum ether extract , chloroform extract , alcohol extract and water extract , obtained by successive solvent extraction of the fruits , indicated the presence of alkaloids , fixed oils and fats , resins , traces of glycosides , proteins and aminoacids, tannins, reducing sugars and sterols and absence of saponins, gums and mucilages. The results of the tests applied are tabulated in Table 1 to 4. Thin-layer chromatography was carried out using Toluene: ethyl acetate (8: 2) as solvent system and visualized the spots in day light by spraying the plate with anisaldehyde - sulphuric acid reagent followed by heating at 120^0 for 10 minutes. One of the yellowish green spot having h*Rf* - value 29 revealed the presence of diosgenin by Co-chromatography using authentic sample while other yellowish green spots having h*Rf*- values 13 and 84, prominent violet spots having h*Rf*- values 91, 53, 43, 34 and 21 and a dark blue spot having h*Rf*- value 14 were also observed in the extract solution as shown in Table 5. The successive solvent extracts of the fruits of *T.terrestris* with petroleum ether, benzene, chloroform, ethanol and water were

scanned at 366 nm by HPTLC using solvent system Toluene: ethyl acetate (8:2), indicated the presence of 5,6,4,4 and 2 components respectively as shown in table 6.

The macroscopic characters (colour, odour , taste , size , shape and surface) of the fruits were observed by naked eyes (Fig.. 10) . Transverse section of the fruit and its powder characteristics were observed under microscope (Fig.s. 11,12 and 13) .

Chicory, *cichorium intybus* Linn. (Fig.. 2) , belongs to the compositae family is locally known as '*Kasni*' , Hakims use seeds, roots and leaves of the plant for the treatment of various ailments.

The proximate analysis of the seeds of the *C. intybus* was carried out to lay certain standards for the air dried drug . The high value of total ash 13.03% indicated the presence of considerable amount of inorganic constituents in the seeds. The petroleum ether-soluble extractive value 4.18% was also rather than high , indicated the presence of fixed oils and fats and sterols etc . The qualitative chemical examination of the petroleum ether extract , alcohol extract and water extract , obtained by successive solvent extraction of the seeds revealed the presence of carbohydrates , phytosterols , proteins and amino acids , tannins , fixed oils and fats and absence of alkaloids , glycosides , saponins , resins , gums and mucilages . The results of the tests applied are tabulated in Table 7 to 10. Thin-layer chromatography of the alcoholic extract of seeds was carried out using chloroform : methanol : formamide (80 : 19 : 1) as solvent system and visualized the spots by spraying the plate with sulphuric acid followed by drying at 75^0 for 3 minutes and their *Rf*-values are recorded in Table 11 . Three substances having *Rf*-values 0.90, 0.86 and 0.83 gave positive Libermann-Burchard test, revealed the presence of three different sterols and other three spots having *Rf*-values 0.36, 0.05 and 0.00 gave positive Molisch's test revealed the presence of three different sugars. Further studies require the

identification of different five phytoconstituents. The successive solvent extracts of the seeds of *C. intybus* with petroleum ether, benzene, chloroform, ethanol and water were scanned by HPTLC using solvent system chloroform : methanol : formamide (80:19:1) indicated the presence of 3,3,3,11 and 10 components respectively as shown in Table 12.

The morphological characters (colour, odour, taste, size, shape and surface) of the seeds were observed by naked eyes (Fig.. 21). Transverse section of the seed and its powder characteristics were observed under microscope (Figs. 22 and 30).

Horse gram, *Dolichos biflorus* Linn. (Fig.. 3) , belongs to the Leguminosae (Papillionaceae) family is popularly known as *'Kulthi'*. It is extensively cultivated and used either as human food (beans or seeds) or as animal fodder (leaves and stem). The seeds have been used in the indigenous system of medicine for a long time as astringent, anthelmintic, nerve tonic, diuretic, aphrodisiac and antipyretic.

The phytochemical studies revealed that there was a high value of total ash 4.07% , indicated the presence of inorganic constituents in the seeds and water-soluble extractive 2.97% , was also high indicated the presence of sugars . The qualitative chemical examination of the petroleum ether extract, alcohol extract and water extract obtained by successive solvent extraction of the seeds revealed the presence of carbohydrates , sterols , proteins and amino acids , fixed oils and fats and absence of alkaloids , glycosides , saponins , tannins , resins , gums and mucilages. The results of the tests applied are tabulated in Tables 13 to 16. Thin-layer chromatography of amino acids of seeds of *Dolichos biflorus* was carried out using n-butanol: acetic acid: water (8: 2: 2) and 96% Ethanol: water (7: 3) as solvent system and visualized the spots by spraying the plate with ninhydrin (0.1% w/v) in butanol. The *Rf*-values of the spots are recorded in Table 17. Eight different amino acids viz.

alanine, histidine, cystine, aspartic acid, leucine, glycine, serine and lysine were identified by Co-chromatography using authentic sample. Thin-layer chromatography of carbohydrates of the seeds was also carried out using Chloroform: methanol (6: 4) and Acetone: water (9: 1) as solvent system and aniline hydrogen phthalate as spraying agent. The Rf-values of the spots are recorded in Table 18. Five different sugars viz. rhamnose, arabinose, fructose, galactose and glucose were identified by Co- chromatography using authentic sample. The successive solvent extracts of the seeds of *D. biflorus* with petroleum ether, benzene, chloroform, ethanol and water along with authentic amino acids and sugars were scanned under UV light using n-butanol : acetic acid : water (8:2:2), 96% ethanol :water (7:3), chloroform : methanol (6:4) and acetone : water (9:1) as solvent systems by HPTLC as shown in Tables 19 and 20.

The morphological characters (colour, odour, taste, size, shape and surface) of the seeds were observed by naked eyes (Fig. 55). Transverse section of the seed and its powder characteristics were observed under microscope (Figs. 56 and 57).

ANTIMICROBIAL ACTIVITY

Results of screening of antibacterial activity and antifungal activity of *Tribulus terrestris* fruit extract are summerised in Table 21 and 22 respectively. It is evident from the results that petroleum ether (60 - 80^0) and ethanol (50%) extracts of the fruits showed significant antibacterial activity against the tested bacterial organisms, i.e., *Staphylococcus aureus* and *Escherichia coli.* The antibacterial activity of both the extracts was compared with Chloramphenicol as antibacterial standard. The petroleum ether (60-80^0) and ethanol (50%) extracts of the fruits also showed significant antifungal activity against *Candida albicans.* The antifungal activity of both the

extracts was compared with Nystatin as antifungal standard.

From these results, it can be concluded that the fruits of *Tribulus terrestris* can be regarded as antimicrobial agent. Further phytochemical studies are needed to identify active constituent(s) responsible for the antimicrobial activity of the fruits.

Antimicrobial activity of petroleum ether and ethanoic extracts of *cichorium intybus* seeds is summerised in Table 23. It is evident from the results that the petroleum ether and ethanolic extracts exhibited moderate to significant antifungal activity against all the tested fungal organisms, i.e., A*spergillus niger, Aspergillus flavus , Candida albicans* and *Fusarium oxysporum* at a concentration of 30 µg and 60 µg . The antifungal activity of both the extracts was compared with Miconazole as antifungal standard at a concentration of 10 µg but none of the extracts was active against all the tested bacterial organisms, i.e., *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* .

From these results, it can be concluded that the seeds of *cichorium intybus* can be regarded as antifungal agent. Further studies require the detailed chemical nature of the active constituent(s) responsible for the antifungal activity of the seeds.

Antimicrobial activity of petroleum ether and ethanolic extracts of *Dolichos biflorus* seeds is summerised in Table 24. It is evident from the results that the ethanolic extract exhibited significant antibacterial activity against all the tested bacterial organisms, i.e., *Bacillus subtilis, Staphylococcus aurecus, Escherichia coli* and *Pseudomonas aeruginosa* at the concentration of 25 µg and 50 µg while the petroleum ether extract at the concentration 50 µg showed a slight antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa.* The antibacterial activity of both the extracts was compared with Ceftazidime as antibacterial standard

at a concentration of 25 µg but none of the extracts was found active against the tested fungal organisms, i.e., *Aspergillus niger* and *Candida albicans.*

From these results, it can be concluded that the seeds of *Dolichos biflorus* can be regarded as antibacterial agent. Further phytochemical studies are needed to identify active constituent(s) responsible for the antibacterial activity of the seeds.

CHAPTER- 5

CONCLUSION

CONCLUSION

India has rich flora of medicinal plants and these medicinal plants are very much used in traditional system of medicine and many pharmacological properties have been attributed to various parts of these plants. Following three plants part have been selected for the standardization and antimicrobial activities.

1. Fruits of *Tribulus terrestris* Linn.
2. Seeds of *Cichorium intybus* Linn.
3. Seeds of *Dolichos biflorus* Linn.

Looking to the medicinal utility of these plants in the literature and comparatively pharmacognostic studies on the parts of these plants are very few and fragmentary. As pharmacognostic screening of the plant parts is essential for identification of the commercial sample, the same has been undertaken to standardize for prevention of admixtures and adulterants in the preparation of Ayurvedic formulation.

Hence the above mentioned parts of these medicinal plants were subjected for standardization and the extracts isolated from these plants part were screened for antimicrobial activities.

The above parts of the plants were procured from local market of Modinagar, Ghaziabad and were identified by Dr. H.B. Naithani , Botanist and Scientist , Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

The proximate analysis of the fruits of *T. terrestris* was carried out to lay certain standards for the air dried drug. The high value of total ash indicated the presence of considerable amount of inorganic constituents in the fruits. The alcohol and water-soluble extractive values were also rather high, indicated the presence of sugars and resin etc. The qualitative examination of the various solvent extracts of fruits indicated the presence of alkaloid, fixed oil, resin, traces of glycosides, proteins,

tannins, reducing sugars and sterols. Thin-layer chromatography indicated the presence of diosgenin by Co-chromatography using authentic sample. Further studies require the identification of other eight phytoconstituents. The successive solvent extracts of the fruits with petroleum ether, benzene, chloroform, ethanol and water when scanned by HPTLC using solvent system toluene: ethyl acetate (8:2) at 366nm, indicated the presence of 5,6,4,4 and 2 components respectively. Macroscopic and microscopic characters of the fruits were also studied.

The proximate analysis of the seeds of *C. intybus* was carried out. The high value of total ash indicated the presence of considerable amount of inorganic constituents in the seeds. The petroleum ether-soluble extractive value was also high, indicated the presence of fixed oil and fat and sterols etc. The phytochemical tests indicated the presence of fixed oil and fat, sterols, carbohydrates, tannins and proteins in various solvent extracts. Thin-layer chromatography study of alcoholic extract showed the presence of three different types of sterols and sugars. Further studies require the identification of different five phytoconstituents. The successive solvent extracts of the seeds with petroleum ether, benzene, chloroform, ethanol and water when scanned by HPTLC using solvent system chloroform : methanol : formamide (8.0 : 1.9 : 0.1) indicated the presence of 3,3,3,11 and 10 components respectively. Macroscopic and microscopic characters of the seeds were also studied.

The proximate analysis of the seeds of *D. biflorus* was carried out. The high value of total ash indicated the presence of considerable amount of inorganic constituents in the seeds. The water-soluble extractive value was also high, indicated the presence of sugars. The qualitative examination of the various solvent extracts of seeds indicated the presence of carbohydrates, sterols, proteins and aminoacids , fixed oil and fat and absence of alkaloids , glycosides , saponins , resins , gums and mucilages . Thin-layer chromatography indicated the presence of eight amino acids viz. alanine, histidine, cystine, aspartic acid, leucine, glycine, serine and lysine as well

as the five various sugars like rhamnose, arabinose, fructose, galactose and glucose by Co-chromatography using authentic sample. The successive solvent extracts of the seeds along with authentic amino acids and sugars were also scanned using different solvent systems by HPTLC. Macroscopic and microscopic characters of the seeds were also studied.

The ethanol extract and petroleum ether (60 - 80°) extract of the fruit of *T. terrestris* possess antimicrobial activity against *Staphylococcus aureus* , *Escherichia coli* and *Candida albicans* as compared to standard drugs. Further studies require the detailed chemical nature of the active principle(s) responsible for the antimicrobial activity.

The antimicrobial studies of the seeds of *C. intybus* revealed that ethanol extract and petroleum ether (60-80°) extract exhibited moderate to significant activity against *Aspergillus niger* , *Aspergillus flavus* , *Candida albicans* and *Fusarium oxysporum* , at a concentration of 30 µg and 60 µg but none of the extracts was active against the tested bacterial organisms. Further the detailed chemical nature of the active principle(s) responsible for the antifungal activity is required.

The ethanol extract of the seeds of *D. biflorus* possesses significant antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at a concentration of 25 µg and 50 µg and petroleum ether extract at the concentration of 50 µg showed a slight antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa.* None of the extracts was found active against *Aspergillus niger* and *Candida albicans.* Further studies require the detailed chemical nature of the active principle(s) responsible for the antibacterial activity.

CHAPTER- 6

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Anonymous , The Wealth of India , Raw Materials , Vol. X , Publication and Information Directorate , CSIR , New Delhi , 1982 , 283.
2. Kirtikar , K.R. and Basu B.D., Indian Medicinal Plants , 2nd Edn., Vol. I , International Book Distributors , Dehradun , 1987, 420.
3. Anonymous , The Wealth of India , Raw Materials , PID , New Delhi , 1992 , 556.
4. Kirtikar, K.R. and Basu, B.D. , Indian Medicinal Plants, 2nd Edn., Vol. II, International Book Distributors , Dehradun , 1988 , 1433.
5. Nadkarni K.M., Indian Materia Medica, 3rd Edn., Vol. I, Popular Prakashan, Bombay, 1993 , 313.
6. Anonymous , The Wealth of India , Raw Materials , Vol. III , PID , New Delhi, 1986 , 101.
7. Zafar , R. , Medicinal Plants of India , C.B.S.Publishers & Distributors , Delhi , 1994 , 70.
8. Kirtikar , K.R. and Basu B.D. , Indian Medicinal Plants , 2nd Edn. , Vol. I , International Book Distributors , Dehradun , 1988 , 805.
9. Nadkarni , K.M. , Indian Materia Medica , Popular Prakashan , Bombay , 3rd Edn., Vol. I , 1993 , 458.
10. Kapoor , L.D. , Ayurvedic Medicinal Plants , CRC Press , Buca Ratou London , New York , Washington , D.C.1001 , 167.
11. Verma , R , *Tribulus terrestris* Linn.- Component of Dashamularishta, *J. Nat. Integ. Med. Ass.* , 1980, 22 (9) , 234.
12. Nath , K. and Malik , N.S. , Chemical composition and nutritive value of *Tribulus terrestris* Linn., *Indian J. Anim. Sci.* , 1970 , 40(4) , 434.

13. Tomowa , M.P. , Panowa , D. and Wulfson , N.S. , Steriod saponines and sapogenins, *Planta Med* , 1974 , 25(3) , 231.

14. Purushothaman , K.K. , Chandrasekharan , S. and Balakrishna , K. , Occurrence of neotigogenin and hecogenin in *Tribulus terrestris* Linn. , *Jour. Res. Ind. Med. Yoga and Homoeo.* , 1976 , 11(1) , 121.

15. Mahato , S.B. , Sahu , N.P. and Pal , B.C. , screening of Tribulus terrestris plants for diosgenin., *J. Inst. Chemists (India)* , 1978 , 50 (10) , 50.

16. Athar , M. and Mahmood , A. , Qualitative estimation of free amino acids from the root nodules of *Tribulus terrestris* Linn. , *Pak J. Bot.* , 1980 12 (10) 91.

17. Duhan , A. , Chauhan , B.M. and Punia , D. , Nutritional values of some non-conventional plant foods of India, *Plant Foods Hum. Nut.* , 1992 , 42 (3) , 193.

18. Africa , B.S. , Protein value of four weeds durinjg fruit setting, *Geobios* , 1980, 7(3) , 121.

19. Saleh , N.A.M. , Ahmed , A.A. and Abdalla , M.F. , Flavonoid glycosides of *Tribulus pentandrus* and *T. terrestris*, *Phytochemistry* , 1982 , 21 (8) , 1995.

20. Singh , S.B. and Thakur , R.S. , Recent advances in the chemistry of steroidal suponins and their genis. *J. Sci. Industr. Res.* , 1983 , 42(6) , 319.

21. Prakash , D. , Singh , P.N. and Wahi , S.P. , Evaluation of *Tribulus terrestris* (Chota gokharu) *Indian Drugs* , 1985 , 22(6) 333.

22. Bourke , C.A. , Stevens , G.R. and Carrigan , M.J. , Locomotor effects in sheep of alkaloids identified in Austrlian *Tribulus terrestris, Aust. , Vet. J.* , 1992 , 69 (7) , 163.

23. Zafar , R. and Aeri , V. , Constituents of *Tribuls terrestries* flowers, *Fitoterapia* , 1992 , 63(1) , 90.

24. Ren , Y. J. and Chen , H.S. , Isolation and identification of a new derivative of Cinnamic amide from *Tribulus terresris, Acta, Pharm. Sinica.*, 1994 , 29 (3), 204.

25. Chakraborty, B. and Neogi , N.C. , Pharmacological properties of *Tribulus terestris* Linn., *Indian J. Pharm. Sci.*, 1978, 40(2), 50.

26. Prakash, D., Singh , P.N. and Wahi , S.P., Evaluation of *Tribulus terrestris* (Chota gokharu), *Indian Drugs* , 1985, 22(6) , 332.

27. Bourke , C.A. Carrigan , M.J. and Dixon , R.J., Upper motor neurone effects in sheep of some beta-carboline alkaloids identified in Zygophyllaceous plants, *Aust. Vet. J.*, 1990, 67(7) , 248.

28. Bourke , C.A. , Carrigan , M.J. and Dixon , R.J. , Locomotor effects in sheep of alkaloids identified in Australian *Tribulus terrestris, Aust. Vet. J.*, 1992 , 69(7) , 163.

29. Anand , R. , Patnaik , G.K. , Kulshreshtha , D.K. and Dhawan , B.N. , Antiurolithiatic activity of *Tribulus terrestris* in albino rats, *Indian J. Pharmacol.*, 1989 , 21(1) , 74.

30. Singh , R.G. , Singh, R.P. , Usha , Shukla , K.P. and Singh , R. , Experimental evaluation of diuretic action of herbal drugs (*Tribulus terrestris* Linn.) on albino rats, *Jour. Res. Edu. Ind. Med.* , 1991 , 10(1) , 19.

31. Anand, R. , Patnaik , G.K. , Kulshreshtha , D.K. and Dhawan , B.N., Activity of certain fractions of *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats, *Indian J. Exp. Biol.,* 1994 , 32 (8), 548.

32. Sangeeta, D. , Sidhu, H., Thind , S.K. and Nath , R., Effect of *Tribulus terrestris* on oxalate metabolism in rats, *J. Ethnopharmacol.*, 1994 , 44(2) , 61.

33. Vijaya , S. and Vasudevan , T.N. , Effect of some medicinal plants on activity of digestive enzymes, *Indian Drugs* , 1994 , 31(5) , 215.

34. Singh, R.H. , Khosa , R.L. and Upadhaya , B.B. , On the antibacterial activity of some Ayurvedic drugs. *Jour. Res. Ind. Med.*, 1974 , 9(2) , 65.

35. Ikram , M. and Haq, I., Screening of medicinal plants for antimicrobial activity, Part I, *Fitoterapia* , 1980 , 51(5) , 231.

36. Jit , S. , Shekhawat , S. , Grover , S. and Nag , T.N., Screening of some plants of Zygophyllaceae for their antimicrobial activity, *Acta Botanica India* , 1986 , 14(1) , 45.

37. Anonymous , The Wealth of India , Raw Materials , Vol. III, Publication and Information Directorate , CSIR , New Delhi , 1992 , 556.

38. Kapoor, L.D., Ayurvedic Medicinal Plants, C.R.C. Press , Boca Raton London, New York , Washington, D.C. , 2001 , 115.

39. Nadkarni K.M., Indian Materia Medica, 3rd Edn. Vol. I, Popular Prakashan, Bombay , 1993 , 313.

40. Barakat, M.Z. , Bassiouni, M. and El-Wakil , M., Ascorbimetry, the determination of ferric iron and cupric copper content of certain vegerables, *J. Sci. Fd. Agric.* , 23 (6) , 1972 , 685.

41. Balbaa, S.I., Zaki , A.Y., Abdel-Wahab , S.M., El-Denshary, E.S.M. and Motazz-Bellah , M., Preliminary phytochemical and pharmacological investigations of the roots of different varieties of *Cichorium intybus*, *Planta Med.*, 1973 , 24(2) , 133.

42. Wight , A.W. and Van Niekerk , P.J. , Determination of reducing sugars, sucrose, and insulin in chicory root by high-performance liquid chromatography, *J. Agric. Food Chem.* , 1983 , 31 (2) , 282.

43. Bridle, P., Loeffler, R.S.T., Timberlake, C.F. and Self , R., Cyanidin 3-malonylglucoside in *Cichorium intybus*, *Phytochemistry* , 1984 , 23(12) , 2968.

44. Takeda, K., Harborne, J.B. and Self, R., Identification and distribution of malonated anthocyanins in plants of the compositae, *Phytochemistry*, 1986 , 25 (6), 1337.

45. Saleem, M., Ahmed, M., Amin, M., Khan, S.A. and Bhatty, M.K., *Cichorium intybus* L.(Kasmi)- total lipid fractions and their fatty acid composition, *Pakistan J. Sci. Ind. Res.*, 1987 , 30(4) , 245.

46. Grayer, R.J. and Harborne, J.B., Survey of antifungal compounds from higher palnts , *Phytochemistry* ,1994 , 37(1) , 32.

47. Park, H.J., Kwon, S.H., Yoo, K.Q., Jung, W.T., Lee, K.T., Kim, J.I. and Han, Y.N., Isolation of magnolialide and artesin from Cichorium intybus revised structure of sesquiterpene lactones, *Nat. Prod. Sci.*, 2000, 6(2), 86.

48. Kisiel, W. and zielinska, K., Guaianolides from *Cichorium intybus* and structure revision of Chchorium sesquiterpene lactones, *Phytochemistry*, 2001, 57(4), 523.

49. Norbaek, R., Nielsen, K. & Kondo, T., Anthocyanins from flowers of *Cichorium intybus*, *Phytochemistry*, 2002, 60(4),357.

50. Pandey V.N., Pharmacological study of some of the cardio-vascular effects of Kutaki, Rohitaka, kakamaci and Kasani used in the management of liver disorders- A preliminary report, *Bull. Medico-Ethno- bot. Res.* 1985, 6 (2-4), 107.

51. Handa, S.S., Sharma, A. and Chakraborti, K.K., Natural products and plants as liver protecting drugs, *Fitoterapia* , 1986, 57(5), 307.

52. Kaur, N., Gupta, A.K. and Saijpaul, B., Hypotriglyceridaemic effect of *Cichorium intybus* roots in ethanol injected and saturated fat fed rats, *J. Ethnopharmacol*, 1988 , 23 (23) 343.

53. Misra, P., Pal, N.L., Guru, P.Y., Katiyar, J.C. and Tandon, J.S., Antimalarial activity of traditional plant against erythrocytic stages of plasmodium berghei, *Int. J. Pharmacog* , 1991, 29(1), 19.

54. Gadgoli, C.H. & Mishra, S.H., Hepatoprotective activity of *Cichorium intybus*, *Indian J. Pharm. Sci.*, 1994, 56(4), 157.

55. Zafar, R. and Ali, S.M., Anti-hepatotoxic effects of root and root callus extract of *Cichorium intybus* Linn., *J. Ethnopharmacol.*, 1998, 63(3), 227.

56. Abou–Jawdah, Y., Sobh, H. and Salameh, A., Antimycotic activities of selected plant flora, growing wild in Lebanon, against phyutopathogenic fungi, *J. Agric. Food Chem.*, 2002, 50 (11), 3208.

57. Anonymous, The Wealth of India, Raw Materials , Vol. III, PID, New Delhi, 1986, 101.

58. Nadkarni , K.M., Indian Materia Medica, Popular Prakashan, Bombay, 3rd Edn., Vol. I , 1993, 458.

59. Zafar, R., Medicinal Plants of India, C.B.S. Publications & Distributors, Delhi 1994, 70.

60. Pant, R. and Tulsiani, D.R.P., Solubility , amino acid composition, and biological evaluation of proteins isolated from leguminous seeds, *J. Agric. Food Chem.*. 1969, 17(2), 361.

61. Mahadevappa, V.G. and Raina, P.L., Nature of some Indian legume lipids, J. *Agric. Food Chem.*, 1978, 26 (5), 1241.

62. Mary, A. and Sastry, K.S., An unusual allantoinase from *Dolichos biflorus*, *Phytochemistry*, 1978, 17(3), 397.

63. Ikram, M.,Review on the medicinal plants, *Hamdard*, 1981, 24(1), 102.

64. Zafar, R. Medicinal Plants of India, C.B.S. Publishers & Distributors, Delhi, 1994, 68.

65. Ingham, J.L., Keen, N.T., Markham, K.R. and Mulheirn,L.J., Dolichins A and B, two pterocarpans from bacteria- treated leaves of *Dolichos biflorus*, *Phytochemistry*, 1981, 20(4), 807.

66. Mitra, J., Das, A. and Joshi, T., Isoflavone diglycoside from the seeds of *Dolichos biflorus*, *Phytochemistry*, 1983, 22(4), 1063.

67. Akihisa, T., Kimura, Y., Roy, K., Ghos, P., Thakur, S. and Tamura, T., Triterpene alcohols and 3-oxocteroids of nine leguminosae seeds, *Phytochemistry*, 1994, 35(5), 1309.

68. Dubey, K.S. and Ansari, A.H., Chemical study of the seeds of *Dolichos biflorus*, *Asian J. Chem.*, 1998, 10(1), 209.

69. Kirtikar K.R. and Basu, B.D. Indian Medicinal Plants, 2nd Edn., Vol. I. International Book Distributors, Dehradun, 1988, 805.

70. Kamboj , V.P. and Dhawan, B.N., Research on plants for fertility regulation in India, *J. Ethnopharmacol*, 1982, 6(2), 191.

71. Laskar , S., Bhattacharyya, U.K. , Sinhababu , A. , Baskar, B.K., Antihepatotoxic activity of Kulthi (*Dolichos biflorus*) seed in rats, *Fitoterapia*, 1998 , 69(5) , 401.

72. Basak, B., Majumdar, S.G., Bhattacharya, U., Laskar, S., Bandopadhyay, A. and Sen, S.K., Effect of different fractions of methanolic extract of the seeds

of *Dolichos biflorus* on some microorganisms, *Hindustan Antibiotics Bull.*, 1992, 34(3) 100.

73. The Indian Pharmacopoecia, 4th Edn., Vol. II, The Controller of Publications, Delhi, 1996, A-47-82.

74. British Pharmacopoeia, Vol. II, Her Majestry's Stationery Office, London , 2000, A-208.

75. Kokate, C.K., Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, Delhi , 1994, 110.

76. Indian Herbal Pharmacopoeia, Vol. II, R.R.L., Jammu Tawi and I.D.M.A., Mumbai, 1999, 159.

77. Stahl, E., Thin-Layer Chromatography, 2nd Edn., Springer-Verlag Berlin, Heidelberg , New York , 1990, 323.

78. Sethi, P.D., High Performance Thin Layer Chromatography, 1st Edn. CBS Publishers and Distributors, New Delhi, 1996,4.

79. Stahl, E., Thin-Layer Chromatography, 2nd Edn., Springer-Verlag Berlin, Heidelberg, New York, 1990, 808.

80. Ibid, 744.

81. Ibid, 813.

82. The Ayurvedic Pharmacopoeia of India, 1st Edn., Part-I, Vol. I, The Controller of Publications, Delhi, 2001, 75.

83. The Indian Pharmacopoeia, 4th Edn., Vol. II, The Controller of Publications, Delhi, 1996, A-105.

84. Ibid, A-106.

LIST OF PUBLICATIONS

Research Papers : 07

1. Gupta, S.K., Sharma, P.K. and Ansari, S.H., (2005), "Standardization of Fruit of *Tribulus terrestris* Linn.", ***Asian J. Chem.***, 17(2), 975-979.

2. Gupta, S.K., Sharma, P.K. and Ansari, S.H., (2005), "Antimicrobial Activity of Fruit of *Tribulus terrestris* Linn", ***Asian J. Chem.***, 17(2), 1355-1356.

3. Gupta, S.K., Sharma, P.K., Ansari, S.H. and Gupta, M.K., (2005), "Standardization of Seeds of *Cichorium intybus* Linn.", ***Asian J. Chem***, 17(4), 2824-2826.

4. Gupta, S.K., Sharma, P.K. and Ansari, S.H., (2005), "Antimicrobial Activity of the Seeds of *Cichorium intybus* Linn.", ***Asian J. Chem***, 17(04), 2839-2840.

5. Gupta, S.K., Sharma, P.K. and Ansari, S.H., (2005), "Standardization of Seeds of *Dolichos biflorus* Linn.", ***Asian J. Chem.***, 17(3), 1809-1812.

6. Gupta, S.K., Sharma, P.K. and Ansari, S.H., (2005), "Antimicrobial Activity of *Dolichos biflorus* Seeds", ***Indian J. Nat. Prod.***, 21(1), 20-21.

7. Gupta, S.K. and Kharya, M.D., (1996), "Phytochemical and Pharmacological studies on Seeds of *Sapindus trifoliatus*", ***Indian J. Nat. Prod.***, 12 (1), 3-8.

Review Articles : 04

1. Gupta, S.K., Zafar, R. and Pathak, D., (1997), "Review on Phytochemical and Pharmacological Aspects of *Tribulus terrestris* Linn.", ***Indian Drugs***, 34(8), 422-426.

2. Gupta, S.K. and Ansari, S.H., (2005), "Review on Phytochemical and Pharmacological Aspects of *Cichorium Intybus* Linn.", ***Asian J. Chem***, 17(1), 33-36.

3. Gupta, S.K. and Sharma, P.K., (2005), "Review on Phytochemical and Pharmacological Aspects of *Dolichos biflorus* Linn.", ***Asian J. Chem.***, 17(1), 37-39.

4. Gupta, S.K. and Zafar, R., (2001), "Review on Phytochemical and Pharmacological Aspects of *Cassia tora* Linn." ***Journal of Science and Pharmacy***, 2(3), 108-110.

Asian Journal of Chemistry Vol. 17, No. 2 (2005), 975–979

Standardization of Fruit of *Tribulus terrestris* Linn.

S.K. GUPTA*, P.K. SHARMA† and S.H. ANSARI‡
Department of Pharmacognosy, Dr. K.N. Modi Institute of Pharmaceutical Education and Research, Modinagar-201 201, India

Different parts of *Tribulus terrestris* Linn. are highly prized remedy amongst the people of India. Since ancient period the fruit is used as demulcent, diuretic, antispasmodic and aphrodisiac. Fruits have been identified by their macroscopic and microscopic characters, cell contents, behaviour of powdered drug with different reagents and preliminary phytochemical analysis.

Key Words: ***Tribulus terrestris* Linn.**

INTRODUCTION

Tribulus terrestris Linn. (Gokhru) is a herbaceous plant belonging to the family Zygophyllaceae. Different parts of the plant, *viz.*, root, leaf and fruit are extensively used in the Indian system of medicine since ancient period. An infusion prepared from fresh leaf and stem is a highly prized remedy amongst the people of Southern India in gonorrhoea and dysuria. The juice of the fruit is an emmenagogue[1–5].

Pharmacognostic reports on the root and fruit of the plant are very few and fragmentary[6, 7]. As pharmacognostic screening of the crude drug is essential for identification of the commercial sample, the same has been undertaken to establish the identifying characters for prevention of admixtures and adulterants in the preparation of Ayurvedic formulation. *T. terrestris* is identified as the smaller variety while a large variety equated with *Pedalium murex* Linn. (Pedaliaceae) is often used as a substitute for the drug.

EXPERIMENTAL

The plant is widely distributed throughout India up to 11000 ft. *T. terrestris* fruits were procured locally from Modinagar market and identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

Macroscopic and microscopic studies were made from free hand. Cell structures of the hard tissues were made by macerating the tissues in conc. HNO_3.

†Institute of Pharmacy, Bundelkhand University, Jhansi, India.
‡Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Humdard, New Delhi-110 062, India.

Powdered drugs were prepared by crushing the fruits in electric grinder. Behaviour of powdered drugs was studied by treating with different chemical reagents. Non-protoplasmic cell contents were studied by treating the sections with chemical reagents. Stains were used and finally mounted either in 50% glycerine or in a mixture of 250% chloral hydrate solution and 50% glycerine solution in the proportion of 9 : 1. Foreign organic matter, moisture content, ash and extractive values, physical data on fruit of *T. terrestris* Linn. were estimated[8]. Preliminary investigations on fluorescence behaviour of ethanol extracts under long (365 nm) and short (257 nm) UV radiation were also studied.

Macroscopic characters: The fruit is pedicellate, globose, 1.3 cm in diameter, 0.8 cm in thickness, possessing five woody, densely hairy, spiny cocci. Each coccus possesses two large sharp, pointed, rigid spines directed towards the apex. The other two smaller, shorter spines are directed downwards. Tips of spines almost meet in pairs together forming pentagonal framework around the fruit. Outer surface of the schizocarp is rough, yellowish, odour faintly aromatic and slightly acrid in taste. Seeds more or less elliptical, tapering at one end, measuring 1.5 × 3.0 mm. seeds several in each coccus, with transverse partitions between them (Fig. 1).

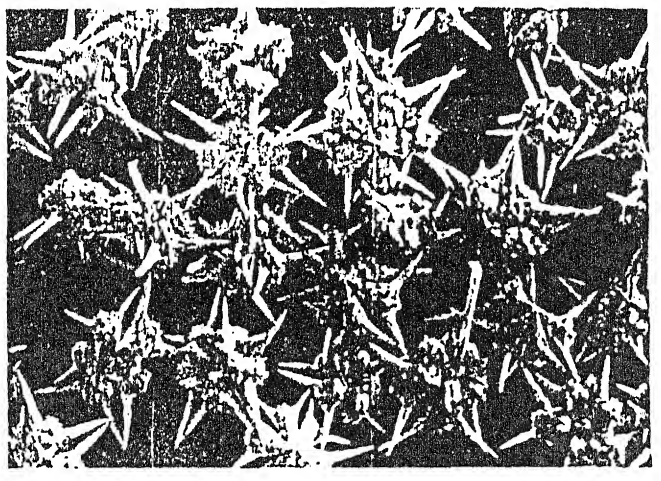


Fig. 1. Fruits of *T. terrestris* Linn

Microscopic Characters: Fruit is very hard, studies are made from macerated tissues as studies from sectional view are not possible. The pericarp is differentiated into epicarp, mesocarp and endocarp. Outer surface of the epicarp is surrounded by non-glandular trichomes. The parenchymatous mesocarp is 6–10 layers thick which embeds calcium oxalate crystals. The sclerenchymatous endocarp is 3–4 layers thick and the cells are compact containing prismatic crystals of calcium oxalate. Fruits are pentalocular, vessels have simple pits and some vessels show helical thickenings. Fibres are lignified, linear, long with tapered ends (Figs. 2 and 3).

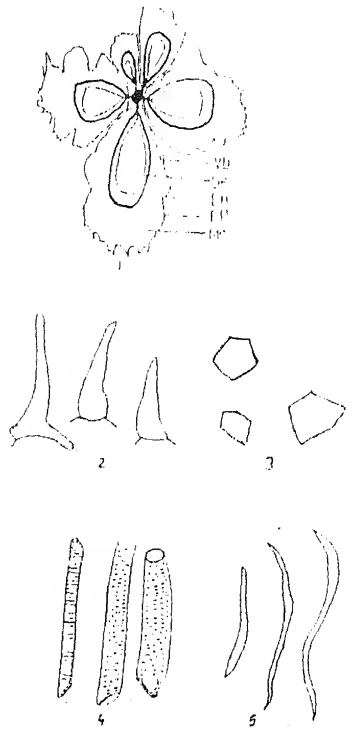


Fig. 2. Microscopical characters of fruit of *T. terrestris* Linn.: (1) T.S. of fruit (diagrammatic), (2) Non-glandular trichomes × 180, (3) Prismatic crystals of calcium oxalate × 720, (4) (i) Vessel showing helical thickening × 367, (ii) Pitted vessels × 367, (5) Fibres × 92

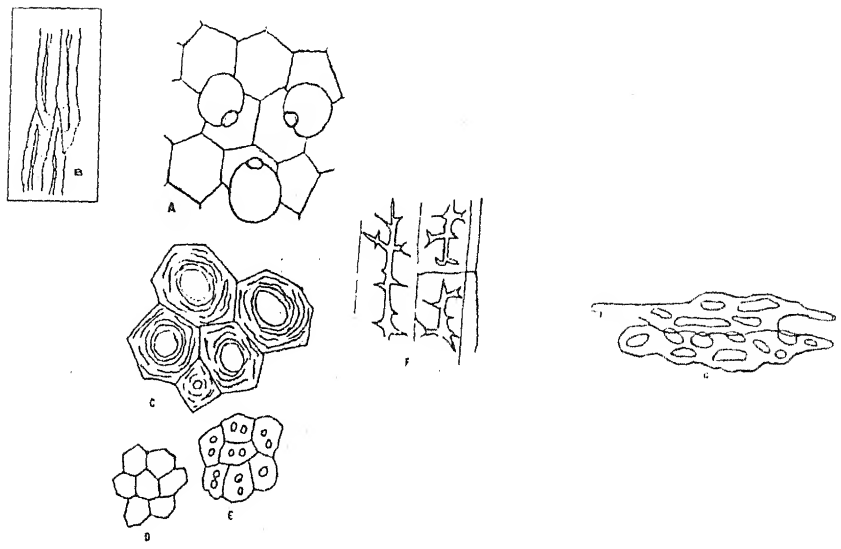


Fig. 3. Microscopical characters of fruit of *T. terrestris* Linn.: (A) Epidermal cells and glands, (B) Bundles of fibres, (C) Cells of outer integument, (D) Cells of inner integument, (E) Endosperm cells with oil drops, (F) Part of sclerenchyma fibres, (G) Reticulated fibres

Physical Constant Values: Foreign organic matter 1.662%; loss on drying 10.10%; total ash 12.79%; acid-insoluble ash 0.97%; sulphated ash 2.07%; water-soluble ash 5.79%; ethanol-soluble extractive 1.862%; water-soluble extractive 16.8%; petroleum ether-soluble extractive 1.018%; chloroform-soluble extractive 1.26%; volatile oil content very small quantity; fluorescent analysis very faint fluorescence in short and long UV light.

Cell Contents: Fats and oil present in the form of globules in the thin-walled cells of the seed; when treated with conc. HCl fat globules are liberated.

REACTION OF POWDERED DRUG WITH DIFFERENT REAGENTS

Water	Powder settles at the bottom producing colourless turbid solution with very little frothing on the surface.
5% KOH	Powder settles at the bottom producing brown colored turbid solution.
Dil. HCl	Powder settles at the bottom producing very faint lemon yellow tinted solution.
Dil. H_2SO_4	— do —
Dil. HNO_3	— do —
$FeCl_3$ solution	Light brown precipitation takes place.
Dragendorf solution	Orange brown coloration and precipitation.
KI and I solution	Light orange brown turbid solution.

Preliminary photochemical analysis: Qualitative examination of the various solvent extracts of fruits indicates the presence of alkaloid, fixed oil, lignin, resin, traces of glycosides, protein, tannins, reducing sugars, sterols and an essential oil[9].

Thin-layer chromatography: 5.0 g sample of powdered fruit was refluxed for 1 h with 50 mL chloroform and filtered. The marc was refluxed for 1 h with 50 mL methanol and filtered. The filtrate was evaporated to dryness under vacuum. 50 mL of 2 N HCl was added to the residue and refluxed for 1 h 1.0 g sodium carbonate was added after cooling the solution and extracted with three successive quantities of 20 mL of chloroform. Combined chloroform layers were washed with water and evaporated to dryness under vacuum. The residue was dissolved in 2 mL of chloroform to be used as test solution.

Test solution and reference solution (1 mg diosgenin in 4 mL methanol) were applied on silica gel G plate, using toluene : ethyl acetate (8 : 2) as solvent system, visualized the spots by spraying the plate with anisaldehyde sulfuric acid reagent and heated at 120°C for 10 min.

A yellowish green spot (R_f 0.29) corresponding to diosgenin was observed in both test and reference solution tracks. Other yellowish green spots (R_f 0.13 and 0.84), prominent violet spots (R_f 0.91, 0.53, 0.43, 0.34 and 0.21) and a dark blue spot (R_f 0.14) were also observed in the test solution[10].

REFERENCES

1. K.R. Kiritikar and B.D. Basu, Indian Medicinal Plants, International Book Distributors, Dehradun, Vol. I, p. 420 (1987).
2. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, Publication and Information Directorate, C.S.I.R., New Delhi, p. 247 (1992).
3. A.K. Nadkarni, Indian Materia Medica, Popular Prakashan, Bombay, p. 1229 (1993).
4. R.N. Chopra, S.L. Nayyar and I.C. Chopra, Chopra's Indigenous Drugs of India, Academic Publishers, Calcutta, p. 430 (1994).
5. The Wealth of India: Raw Materials, NISCOM, C.S.I.R., New Delhi, Vol. 10, p. 283 (1998).
6. The Ayurvedic Pharmacopoeia of India, Part I, Vol. I, 1st Edn.,Govt. of India, Ministry of Health and Family Welfare, New Delhi, p. 38 (2001).
7. R. Zafar, Medicinal Plants of India, CBS Publishers and Distributors, Delhi, pp. 121–132 (1994).
8. Pharmacopoeia of India, Vol. II, The Controller of Publications, New Delhi, pp. A-47–89 (1996).
9. C.K. Kokate, Practical Pharmacognosy, 4th edn., Vallabh Prakashan, New Delhi, p. 110 (1994).
10. Indian Herbal Pharmacopoeia, Vol. II, R.R.L., Jammu Tawi and I.D.M.A., Mumbai, p. 154 (1999).

(*Received*: 17 June 2004; *Accepted*: 24 December 2004) AJC-4036

207th MEETING OF THE ELECTROCHEMICAL SOCIETY

QUEBEC CITY, CANADA

15–20 MAY 2005

Contact:
Erin Goodwin
The Electrochemical Society, Inc.
65 South Main Street, Building D
Pennington, NJ 08534-2839, USA
Tel.: +1(609)737-1902
Fax: +1(609)737-2743
E-mail: erin.goodwin@electrochem.org
Website: http://www.electrochem.org/meetings/future/207/meeting.htm

Asian Journal of Chemistry Vol. 17, No. 2 (2005), 980–984

Synthesis and Antimicrobial Activity of New 3-Amino sulphonyl[3′-chloro-4′-(substituted phenyl)-2′-oxo azetidine]indole

K. MURUGESH†, V.S. SARAVANAN†, J. THOMAS LEONARD† and G. SONIA*
Department of Medicinal Chemistry, R.V.S. College of Pharmaceutical Sciences Coimbatore-641 042, India

A series of new 3-amino sulphonyl[3′-chloro-4′-(substituted phenyl)-2′-oxo azetidine]indole derivatives (**5a–j**) have been prepared from the respective 3-(substituted benzylidene hydrazine) sulphonyl indoles (**4a–j**) by treating with chloroacetyl chloride in presence of ethanol. The required substituted benzylidene hydrazino sulphonyl indoles (**4a–j**) were obtained from hydrazino sulphonyl indole (**3**) by condensing with appropriate aromatic aldehydes. Chloro-sulphonyl indole (**2**) when treated with hydrazine yielded the respective hydrazides. 3-Aminosulphonyl [3′-chloro-4′-(4-nitro phenyl)-2′-oxo azetidine]indole showed moderate to good antimicrobial activity.

Key Words: Indole derivatives, Azetidine, Antimicrobial activity.

INTRODUCTION

Substituted indoles are associated with psychotropic[1], antiinflammatory[2–5], CNS depressant, anticonvulsant and antimicrobial activities. The azetidinone moiety is known to potentiate the biological activity[6–8]. Azetidinone containing fused indole moieties are likely to be shown enhanced biological activities.

EXPERIMENTAL

Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on Shimadzu FTIR-3000 instrument, ^{1}H NMR spectra were recorded on Bruker 300 MHz spectrophotometer using TMS as an internal standard. The purity of synthesized compounds was routinely checked by TLC.

Synthesis of 3-chloro sulphonyl indole (2)

Equimolar proportions of indole (0.01 mol) and chlorosulphonic acid was added drop by drop and it was shaken from time to time to ensure thorough

†Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.
Email: sarugesh@yahoo.com

Asian Journal of Chemistry Vol. 17, No. 2 (2005), 1355–1356

NOTE

Antimicrobial Activity of Fruit of *Tribulus Terrestris* Linn.

S.K. GUPTA*, P.K. SHARMA† and S.H. ANSARI‡
Department of Pharmacognosy
Dr. K.N. Modi Institute of Pharmaceutical Education and Research
Modinagar-201 201, India

The *in-vitro* antimicrobial activity of the fruit of *Tribulus terrestris* has been studied using petroleum ether (60–80°C) and ethanolic (50%) extracts against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Both the extracts showed significant activity against all test micro-organisms.

Key Words: *Tribulus Terrestris* Linn., Antimicrobial.

Tribulus terrestris (Zygophyllaceae), commonly known as 'Chota-gokhru', is an annual or perennial plant growing throughout India[1]. It is described as a highly valuable drug used to restore the depressed liver for the treatment of fullness in the chest and mastitis and also used to dispel the wind and clear the eyes for the treatment of acute conjuctivitis, headache and vertigo. *Tribulus terrestris* is also reported to have antimicrobial, antihypertension, diuretic, antiacetylcholine and haemolytic activity and to stimulate spermatogenesis and libido[2-7]. The current study was undertaken to evaluate the antimicrobial activity of *Tribulus terrestris* fruit extract.

The drug *T. terrestris* fruits were purchased from the local drug market of Modinagar. The drug was identified and authenticated by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

The air dried, pulverized fruits of *T. terrestris* were exhaustively extracted with ethanol (50%) and petroleum ether (60–80°C) using Soxhlet extractor and concentrated under reduced pressure. The concentrated ethanol extract and petroleum extract were dissolved in dimethyl sulfoxide (DMSO), an inert solvent which was also used as control and found inert against all the tested micro-organisms.

The growth medium used for the test micro-organisms, *viz.*, *Staphylococcus aureus* and *Escherichia coli,* was medium No. 1 (Hi-Media) and for *Candida albicans* Sabouraud dextrose agar (Hi-Media). The petri plates were pre-seeded with 10 mL of growth medium and 4 mL of inoculum in case of *E. coli* and *S. aureus* and 6.5 mL of inoculum in case of *C. albicans*. Paper discs of 6 mm diameter which absorb 0.1 mL of extract (ethanol/pet. ether) and known quantity of standard reference antibiotics were used for comparison of zone of inhibition.

†Institute of Pharmacy, Bundelkhand University, Jhansi, India.
‡Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India.

The inoculated bacterial cultures were incubated at 32–35°C for 21 h and fungus culture at 22–25°C for 48 h. The antimicrobial activity was assayed by disc diffusion method[8]. The zone of inhibition was measured and average of the independent determinations was recorded (Tables 1 and 2).

It is evident from the results that the ethanol extract and pet.-ether (60–80°C) extract of fruit of *Tribulus terrestris* showed positive response against organisms tested as compared to standard drug, chloramphenicol.

The chemical nature of the active principles responsible for the antimicrobial activity of the fruit extracts was not established.

TABLE-1
ANTIBACTERIAL ACTIVITY OF *TRIBULUS TERRESTRIS* FRUIT EXTRACT

Extract/Antibiotic	Concentration (μg per mL)	Diameter of the zone of inhibition (mm)	
		S. aureus	*E. coli*
Pet.-ether(60–80°C) extract	20	8.8	9.2
Ethanol (50%) extract	20	10.5	9.0
Chloramphenicol	30	17.0	15.0

TABLE-2
ANTIFUNGAL ACTIVITY OF *TRIBULUS TERRESTRIS* FRUIT EXTRACT

Extract/Antibiotic	Concentration (μg per mL)	Diameter of the zone of inhibition (mm)
		Candida albicans
Pet.-ether(60–80°C) extract	75	17.9
Ethanol (50%) extract	75	16.8
Chloramphenicol	100 units	21.0

REFERENCES

1. R.N. Chopra, Chopra's Indigenous Drugs of India, Academic Publisher, Calcutta, p. 430 (1994).
2. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Book Distributors, Dehradun, Vol. I, pp. 420–422 (1987).
3. A.K. Nadkarni, Indian Materia Medica, Popular Prakashan (P) Ltd., Bombay, pp. 1229–1232 (1993).
4. R. Zafar, Medicinal Plants of India, CBS Publishers and Distributors, Delhi, pp. 127–129 (1994).
5. R.H. Singh, R.L. Khosa and B.B. Upadhaya, *J. Res. Indian Med.*, **9,** 65 (1974).
6. M. Ikram and I. Haq, *Fitoterapia*, **51,** 231 (1980).
7. T.S. Wu, L.S. Shi and S.C. Kuo, *Phytochemistry*, **50,** 1411 (1999).
8. Indian Pharmacopoeia, Controller of Publications, Delhi, Vol. II, p. A-105 (1996).

(*Received:* 10 September 2004; *Accepted:* 6 December 2004) AJC-4105

Asian Journal of Chemistry Vol. 17, No. 4 (2005), 2824–2826

NOTE

Standardization of Seeds of *Cichorium intybus* Linn.

S.K. GUPTA*, P.K. SHARMA†, S.H. ANSARI‡ and M.K. GUPTA†
Department of Pharmacognosy, Dr. K.N. Modi Institute of Pharmaceutical Education and Research, Modinagar-201 201, India

Seeds of *Cichorium intybus* Linn. are tonic to the brain, alexiteric, appetizer and useful in headache, ophthalmia, biliousness, lumbago, troubles of the spleen and asthma. The present work attempts to summarize the pharmacognostical characters of the seed.

Key Words: *Cichorium intybus.*

Cichorium intybus Linn. (fam. Compositae) is locally known as Kasni. It is an erect perennial herb and cultivated throughout India, also grows wild in Punjab, northwest India and Hyderabad in areas up to 1,800 m elevation. The seeds are reported to be carminative and cordial. A decoction is used in obstructed menstruation and for checking bilious vomiting[1–3].

The present investigation was undertaken to standardize the seeds of *Cichorium intybus* by carrying out various pharmacognostical characteristics for prevention of adulterants in Ayurvedic formulation.

The seeds of *Cichorium intybus* were procured locally from Modinagar market and were identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

The morphological characters (colour, odour, size, shape, surface and taste) of the seeds were observed. Foreign organic matter, loss on drying, ash values, extractive values and other physical parameters were determined by pharmacopoeal methods[4]. The behaviour of the powdered seeds with different chemical reagents and fluorescence characters of the alcoholic extract under UV radiation (254 and 366 nm) were also observed. The petroleum ether, ethanol and distilled water extracts were subjected to various chemical tests for the identification of phytoconstituents[5] and ethanolic extract was subjected to thin layer chromatography[6].

Observation

Seeds are rough, oval in shape, bland in taste, odourless and light brown to pale brown in colour, having a size of about 3–4 mm long and 2–3 mm wide.

†Institute of Pharmacy, Bundelkhand University, Jhansi-284 128, India.
‡Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India.

Physical constant values

Foreign organic matter: 0.67%; Loss on drying: 9.11%; Total ash: 13.03%; Acid-insoluble ash: 1.90%; Sulphated ash: 12.33%; Water-soluble ash: 2.61%; Ethanol-soluble extractive: 1.04%; Water-soluble extractive: 2.25%; Petroleum ether-soluble extractive: 4.18%; Chloroform-soluble extractive: 0.98%; Volatile oil content: Nil; Fluorescent analysis: Very faint fluorescence in short and long UV light.

Cell contents

Fat and oil present in the form of globules in the thin-walled cells of the seed; when treated with conc. HCl fat globules are liberated.

Behaviour of powdered seed with different reagents

Water and 5% KOH	–	Powder settles at the bottom producing light greyish brown coloured turbid solution.
Dil. HCl, dil. H_2SO_4 and dil. HNO_3	–	Powder settles at the bottom producing clear solution.
$FeCl_3$ soln. and Dragendorff's soln.	–	Powder settles at the bottom. Some powder floats producing clear orange liquid.
KI and I_2 soln.	–	Powder settles at the bottom producing reddish brown clear liquid.

Preliminary phytochemical analysis: Qualitative examination of the various solvent extracts of seeds revealed the presence of fixed oil and fat, carbohydrates, proteins, tannins and sterols and absence of alkaloids and saponins.

Thin-layer chromatography: Seeds powder (140 g) was extracted with ethyl alcohol in a Soxhlet extractor for 18 h and concentrated under reduced pressure at low temperature (45–50°C). The extract was subjected to thin-layer chromatography using TLC aluminium sheets (Merck), previously activated by heating at 110°C for 30 min. Several solvent systems were tried. The best separation was achieved by the solvent system chloroform : methanol : formamide (80 : 19 : 1) for half an hour, drying in an oven at 110°C for 15 min, seen in UV light and then sprayed with Liebermann-Burchard reagent, Molisch's reagent and with suphuric acid, separately. Observations are given in Table-1.

Three spots (R_f 0.83, 0.86 and 0.90) gave positive Liebermann-Burchard test and other three spots having the R_f values 0.36, 0.05 and 0.00 showed pale blue, pale blue and green fluorescence, respectively in UV light, gave positive Molisch's test.

The phytochemical tests indicated the presence of fixed oil and fat and sterols in petroleum ether extract; carbohydrates, sterols, tannins and proteins in ethanolic extract; and carbohydrates, tannins and proteins in distilled water extract. Chromatography study shows the presence of three different types of sterols and sugars in ethanolic extract.

TABLE-1
TLC OF ALCOHOLIC EXTRACT OF SEEDS OF *CICHORIUM INTYBUS* AND RESULTS OBTAINED BY DIFFERENT REAGENTS

S.No. of spots	R_f values	UV light	Sulphuric acid	Liebermann-Burchard reagent	Molisch's reagent
1	0.98	—	Violet-blue	–	–
2	0.90	—	Violet	+	–
3	0.86	—	Blue	+	–
4	0.83	—	Purple	+	–
5	0.73	Violet	—	–	–
6	0.66	—	Red	–	–
7	0.60	—	Blue	–	–
8	0.47	—	Pale violet	–	–
9	0.36	Pale blue	Dirty green	–	+
10	0.05	Pale blue	with violet	–	+
11	Zero	Green	tinge	–	+

The present study can help in authenticating the seeds prior to Ayurvdic formulation.

REFERENCES

1. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Book Distributors, Dehradun, Vol. II, p. 1433 (1988).
2. Anonymous, The Wealth of India, Raw Materials, CSIR, New Delhi, Vol. III, p. 561 (1992).
3. R.N. Chopra, I.C. Chopra, K.L. Handa and L.D. Kapur, Chopra's Indigenous Drugs of India, Academic Publishers, Calcutta, p. 319 (1994).
4. Indian Pharmacopoeia, The Controller of Publications, Delhi, Vol. II, p. A-53, 54, 89 (1996).
5. C.K. Korate, Practical Pharmacognosy, Vallabh Prakashan, New Delhi, p. 110 (1994).
6. E. Stahl, Thin-Layer Chromatography, Springer-Verlag Berlin-Heidelberg-New York, p. 808 (1990).

(*Received*: 13 November 2004; *Accepted*: 22 July 2005) AJC-4339

Asian Journal of Chemistry Vol. 17, No. 4 (2005), 2839–2840

NOTE

Antimicrobial Activity of the Seeds of *Cichorium intybus* Linn.

S.K. GUPTA*, P.K. SHARMA† and S.H. ANSARI‡
Department of Pharmacognosy, Dr. K.N. Modi Institute of Pharmaceutical Education and Research, Modinagar-201 201, India

The antimicrobial activity of petroleum ether and ethanolic extracts of the seeds of *Cichorium intybus* Linn. has been studied against various microorganisms by disc diffusion method. Both the extracts at a concentration of 30 and 60 µg/disc showed significant activity against the fungal organisms investigated.

Key Words: Antimicrobial activity, *Cichorium intybus*.

Cichorium intybus Linn. (fam. Compositae) is locally known as 'Kasni'. Various medicinal properties have been attributed to this plant in the traditional system of Indian medicine. The seeds are reported to be carminative, cordial, a brain tonic and useful in headache, ophthalmia, throat inflammation, lumbago, enlargement of the spleen and asthma. A decoction is used in obstructed menstruation and for checking bilious vomiting[1–4].

The dried seeds of *Cichorium intybus* were procured locally from the Modinagar market and identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

The seed powder (500 g) was extracted with petroleum ether (60–80°C) and ethanol (95%) successively in a Soxhlet extractor and the extracts were concentrated to dryness *in vacuo*. Antimicrobial activity of the extracts was determined using paper-disc diffusion method[5] by measuring the zone of inhibition. The extracts at a concentration of 30 µg and 60 µg/disc were screened for their antimicrobial activity using *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus, Candida albicans* and *Fusarium oxysporum* as test organisms.

Nutrient agar (Hi Media) and sabouraud dextrose agar (Hi Media) were used as media for bacteria and fungi respectively. Control experiment was carried out under similar condition by using ceftazidime and miconazole as a standard for antibacterial and antifungal activity, respectively. The petri dishes were incubated at 37°C for 48 h. The zones of inhibition are recorded in Table-1.

†Institute of Pharmacy, Bundelkhand University, Jhansi-284128, India.
‡Faculty of Pharmacy, Jamia Hamdard, New Delhi-62, India.

TABLE-1
ANTIMICROBIAL ACTIVITY OF PETROLEUM ETHER AND ETHANOLIC EXTRACTS OF *CICHORIUM INTYBUS* SEEDS

Microorganisms	Pet. ether (µg/disc)		Ethanol (µg/disc)		Ceftazidime (µg/disc)	Miconazole (µg/disc)
	30	60	30	60	30	10
Bacteria:						
Bacillus subtilis	–	+	–	–	+++	NT
Staphylococcus aureus	–	–	+	+	+++	NT
Escherichia coli	–	+	–	–	++	NT
Pseudomonas aeruginosa	–	–	–	+	+++	NT
Fungi:						
Aspergillus niger	+	++	++	+++	NT	+++
Aspergillus flavus	++	+++	++	++	NT	+++
Candida albicans	+	++	++	+++	NT	+++
Fusarium oxysporum	++	+++	+	++	NT	+++

Disc diameter = 4 mm; Zone of inhibition (mm): < 4; + = 5–10; ++ = 11–15; +++ = >16; NT = not tested.

The study reveals that petroleum ether and ethanolic extracts exhibited moderate to significant activity against all the tested fungal organisms at the concentration of 30 and 60 µg but none of the extracts was active against the tested bacterial organisms.

REFERENCES

1. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Book Distributors, Dehradun, Vol. II, p. 1434 (1988).
2. Anonymous, The Wealth of India: Raw Materials, C.S.I.R., New Delhi, Vol. III, p. 561 (1992).
3. R.N. Chopra, I.C. Chopra, K.L. Handa and L.D. Kapur, Chopra's Indigenous Drugs of India, Academic Publishers, Calcutta, p. 318 (1994).
4. K.M. Nadkarni, Indian Materia Medica, Popular Prakashan, Bombay, Vol. I, p. 314 (1993).
5. Indian Pharmacopoeia, The Controller of Publications, Delhi, Vol. II, p. A-106 (1996).

(*Received*: 18 December 2004; *Accepted*: 22 July 2005) AJC-4344

Asian Journal of Chemistry Vol. 17, No. 3 (2005), 1809–1812

Standardization of Seeds of *Dolichos Biflorus* Linn.

S.K. GUPTA*, P.K. SHARMA† and S.H. ANSARI‡
Department of Pharmacognosy, K.N. Modi Institute of Pharmaceutical Education and Research, Modinagar-201 201, India

Seeds of *Dolichos biflorus* Linn. are considered to be very useful for removing kidney stones. These are used as astringent, diuretic and tonic. Seeds have been identified by their macroscopic and microscopic characters, cell contents, behaviour of powdered drug with different reagents and preliminary phytochemical analysis.

Key Words: Standardization, Seeds, *Dolichos biflorus* Linn.

INTRODUCTION

Dolichos biflorus Linn. (Fam. Leguminosae) is also known as horse gram and *Kulthi* in Hindi. It is a native of Southeast Asia, throughout the tropics, India, Malaysia and West Indies. About 14 species occur in India, of which *D. biflorus* and *D. lablab* are extensively cultivated and used either as human food (beans or seeds) or as animal fodder (leaves and stem). Seeds extract seem to be useful for the patients suffering from urinary or kidney troubles, eye troubles, piles, enlargement of the spleen and pain in the liver[1–5].

EXPERIMENTAL

The seeds of *Dolichos biflorus* were procured locally from Modinagar market and identified by Dr. H.B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

Macroscopic and microscopic studies were made from free hand. Seeds were powdered by crushing in electric grinder. Behaviour of powdered drugs was studied by treating with different chemical reagents. Foreign organic matter, loss on drying, ash values, extractive values and other physical parameters on seeds of *D. biflorus* Linn. were determined as per I.P. Methods[6]. Preliminary investigations on fluorescence behaviour of ethanol extracts under long (365 nm) and short (257 nm) UV radiation were also studied.

RESULTS AND DISCUSSION

Macroscopic Characters: Fruits contain 5–7 seeds, compressed, hard, surface smooth, ellipsoid, flattened, 4–6 mm long and 4 mm wide, micropyle prominent, greyish to reddish brown in colour (Fig. 1).

†Institute of Pharmacy, Bundelkhand University, Jhansi-248 128, India.
‡Faculty of Pharmacy, Jamia Hamdard, New Delhi, 110 062, India.

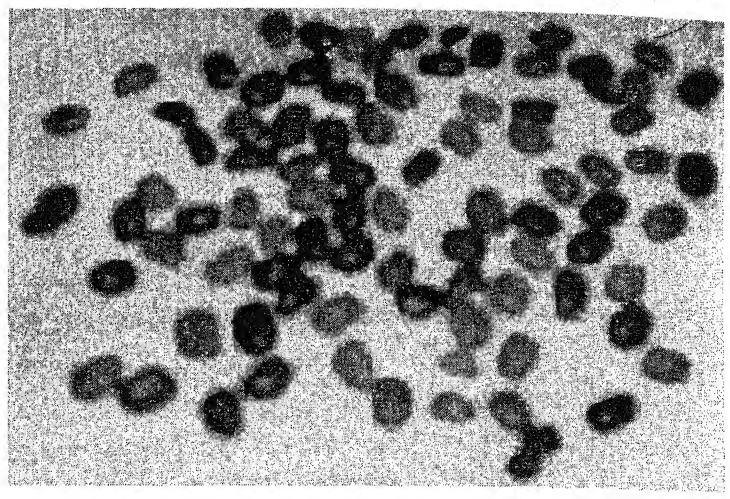


Fig. 1. Seeds of *Dolichos biflorus* Linn.

Microscopic Characters: Transverse section of seed shows testa consisting of a single layer of columnar, thin-walled, parenchymatous, palisade like cells covered with a thin cuticle followed by single layer of rectangular to square bearer cells and 3–4 layers of thin-walled rectangular parenchymatous cells, more wide at micropyler region; cotyledon consisting of single layer of upper and lower epidermis covered with a thin cuticle; epidermal cells thin-walled, rectangular and parenchymatous followed by mesophyll, consisting of angular parenchymatous cells, filled with numerous simple starch grains and protein bodies also present. Powder is whitish in colour, consisting of broken pieces of testa, parenchymatous cells and startch (Fig. 2).

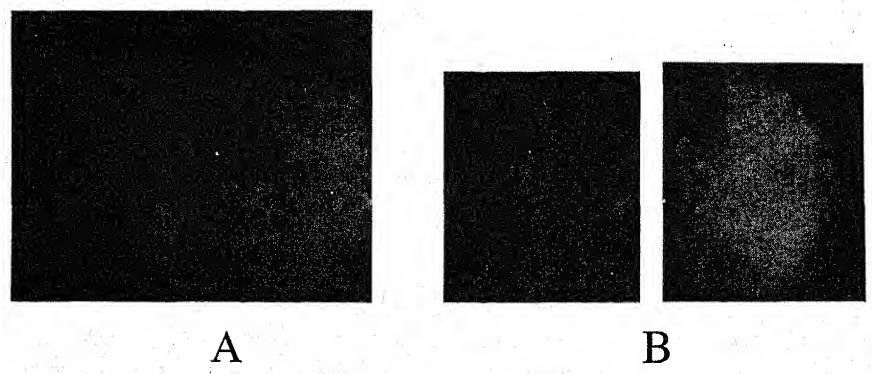


Fig. 2. Microscopic characters of seeds of *Dolichos biflorus* Linn.: (A) T.S. of seed (cellular) × 100, (B) Powder characteristics × 100

Physical Constant Values

Foreign organic matter : Nil, Loss on drying : (10.9%); Total ash : (4.07%); Acid-insoluble ash : (0.80%); Sulphated ash : (8.38%); Water-soluble ash : (2.97%); Ethanol-soluble extractive : (0.48%); Water-soluble extractive : (5.16%); Petroleum ether-soluble extractive : (1.56%); Chloroform-soluble extractive : 0.23% Volatile oil content : (Nil); Fluorescent analysis : Very faint fluorescence in short and long UV light

Cell Contents: Fats and oil present in the form of globules in the thin-walled cells of the seed when treated with conc. HCl fat globules are liberated.

Reaction of powdered drug with different reagents

Water	:	Powder settles at the bottom producing dark grey brown coloured turbid solution with very little frothing on the surface.
5% KOH	:	Powder settles at the bottom producing greenish coloured turbish solution.
Dil. HCl	:	Powder settles at the bottom producing clear solution
Dil. H_2SO_4	:	—do—
Dil. HNO_3	:	—do—
$FeCl_3$ soln.	:	Powder settles at the bottom producing clear orange liquid
Dragendorff's soln.	:	—do—
KI and I soln.	:	Powder settles at the bottom producing reddish brown clear liquid.

Preliminary phytochemical analysis

Qualitative examination of the various solvent extracts of seeds indicates the presence of fixed oil, carbohydrate, protein, fat and sterols[7].

Thin-layer chromatography

Part I: Seeds powder was defatted with petroleum ether (60–80°C) in soxhlet extractor. 1.0 g of defatted seed powder was warmed with 10 mL ethanol (70% v/v) for 30 min and centrifuged. The residue was re-extracted with ethanol and centrifuged. This process was repeated (8–9 times) till the supernatant was negative to ninhydrin test. All the supernatants were combined and evaporated to dryness *in vacuo*, dissolved in 0.5–1.0 mL distilled water and centrifuged. The clear supernatant was subjected to thin-layer chromatography by using TLC aluminium sheets (Merck). *n*-Butanol : acetic acid : water and 96% ethanol : water were used as mobile phase. The chromatograms were sprayed with ninhydrin (0.1% w/v) in butanol. Observations are given in Table-1.

TABLE-1
SOLVENT SYSTEM

S.No.	*n*-Butanol : acetic acid : water (8 : 2 : 2)		96% ethanol : water (7 : 3)		Amino acids identified
	R_f reported[8]	R_f found	R_f reported[8]	R_f found	
1.	0.22	0.22	—	—	Alanine
2.	0.05	0.06	0.33	0.33	Histidine
3.	0.09	0.09	0.39	0.39	Cystine
4.	0.17	0.17	0.55	0.55	Aspartic acid
5.	0.44	0.45	0.61	0.60	Leucine
6.	0.18	0.18	0.43	0.42	Glycine
7.	—	—	0.48	0.48	Serine
8.	0.03	0.03	0.03	0.03	Lysine

Part II: The defalted seeds were extracted with water and concentrated to dark brown mass. It was found to respond to positive tests for sugars which were identified by thin-layer chromatography on silica gel G, impregnated with sodium acetate buffer using (i) chloroform : methanol, (ii) acetone : water as solvent system and aniline hydrogen phthalate as spraying reagent. Observations are given in Table-2.

TABLE-2
SOLVENT SYSTEM

S.No.	Chloroform : methanol (6 : 4)		Acetone : water (9 : 1)		Sugars identified
	R_f reported[8]	R_f found	R_f reported[8]	R_f found	
1.	0.54	0.53	0.71	0.72	Rhamnose
2.	0.41	0.41	0.53	0.53	Arabinose
3.	0.30	0.29	0.47	0.48	Fructose
4.	0.27	0.27	0.45	0.45	Galactose
5.	0.37	0.36	0.55	0.56	Glucose

REFERENCES

1. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, C.S.I.R., New Delhi, p. 100 (1992).
2. Anonymous, The Wealth of India, Raw Materials, C.S.I.R., New Delhi, Vol. III, p. 101 (1952).
3. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Book Distributors, Dehradun, Vol. I, p. 805 (1987).
4. R. Zafar, Medicinal Plants of India, CBS Publishers and Distributors, Delhi, p. 62 (1994).
5. S.S. Handa and M.K. Kaul, Supplement to Cultivation and Utilization of Medicinal Plants, R.R.L., Jammu, p. 43 (1996).
6. Indian Pharmacopoeia, Controller of Publications, Delhi, Vol. II, p. A-53, 54, 89 (1996).
7. C.K. Kokate, Practical Phamacognosy, Vallabh Prakashan, New Delhi, p. 110 (1994).
8. E. Stahl, Thin-Layer Chromatography, Springer-Verlag, Berlin-Heidelberg-New York, p. 744, 813 (1990).

(*Received*: 5 October 2004; *Accepted:* 5 April) AJC-4171

ANTIMICROBIAL ACTIVITY OF *DOLICHOS BIFLORUS* SEEDS

S.K. Gupta[1*], P.K. Sharma [2] and S.H. Ansari[3]
Dr. K.N. Modi Institute of Pharmaceutical Education and Research,
Modinagar[1] - 201 201
Institute of Pharmacy, Bundelkhand University, Jhansi[2] - 284 128
and
Faculty of Pharmacy, Jamia Hamdard, New Delhi[3]- 110 062

ABSTRACT

The antimicrobial activity of the seeds of *Dolichos biflorus* has been studied using petroleum ether and ethanol extracts against various micro-organisms by disc diffusion method. The ethanol extract at a concentration of 25 and 50 µg/disc showed significant activity against the bacterial organisms investigated.

Dolichos biflorus Linn (Leguminosae) is also known as horse gram. Seed extract is useful for the patients suffering from urinary or kidney troubles, eye troubles, piles, enlargement of the spleen and pain in the liver[1-7].

The seeds of *Dolichos biflorus* were procured locally from Modinagar market and identified by Dr H. B. Naithani, Botanist and Scientist, Forest Tree Seed Laboratory, Silviculture Division, Forest Research Institute, Dehradun.

Antimicrobial Activity

Seed powder (500 g) was successively extracted with petroleum ether (60-80 °C) and ethanol (95%) in a Soxhlet extractor. The extracts were concentrated to dryness *in vacuo.* The antimicrobial activity of the extracts was evaluated by disc diffusion method[8]. Both the extracts at a concentration of 25 µg and 50 µg were screened for their antimicrobial activity using *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger* and *Candida albicans* as test organisms.

The Ceftazidime and Ketoconazole were used as standard for antibacterial and antifungal activity respectively. Nutrient agar (Hi Media) and Sabouraud dextrose agar (Hi Media) were used as media for bacteria and fungi respectively. The plates were incubated at 37 °C for 48 hrs. for bacteria and at 26 ± 1°C for 72 hrs. for fungi. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the disc.

The study reveals that the ethanol extract exhibited significant activity against all the tested bacterial organisms at the concentration of 25 µg and 50 µg. The petroleum ether extract at the concentration of 50 µg showed a slight antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa.* None of the extracts were found active against the tested fungal organisms.

REFERENCES

1. Chopra, R.N., Nayar, S.L. and Chopra, I.C., "Glossary of Indian Medicinal Plants", CSIR, New Delhi, 1992, p. 100.

2. "The Wealth of India-Raw Materials", Vol. III, CSIR, New Delhi, 1952, p. 101.

* For correspondence

PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON SEEDS OF *SAPINDUS TRIFOLIATUS*

S.K. Gupta and M.D. Kharya*
Dr. K.N. Modi Institute of Pharmaceutical Education and Research,
Modinagar - 201204.

ABSTRACT

Physico-chemical characteristics of fixed oil and fatty acids of the seed kernels of *Sapindus trifoliatus* were determined. Three out of five fatty acids were indentified to be palmitic, stearic and oleic acids. One out of two unsaponifiable components was identified to be ß-sitosterol. Unsaponifiable matter showed increase in force of contraction on frog's heart and slight protection against electro-shock induced convulsions.

INTRODUCTION

Different parts of the *Sapindus trifoliatus* Linn. (Sapindaceae), also known as Indian Soap-nut, are mentioned in indigenous systems of medicine because of their therapeutic values. Pessaries made out of the seed kernels are used in amenorrhoea and to stimulate the uterus facilitating child birth[1]. The seed oil is employed medicinally as well as in the manufacture of soap[2]. Seed kernels contain 44.7% of a non-drying fatty oil comprising olein (61.5%), eicosanin (21.9%), stearin (8.5%), palmitin (5.6%) and lignocerin (2.5%). Various physico-chemical characteristics of phospholipid fraction of seed-oil have been reported [3-5].

Considering these reports on the medical utility of the *S. trifoliatus* an attempt was made for systemic phytochemical and pharmacological investigations of its seeds.

EXPERIMENTAL

Proximate analysis and successive solvent extraction of the authenticated market seeds of *S. trifoliatus* leading to qualitative tests for various constituents was taken up. Fixed oil from seed kernels was extracted and studied for its physico-chemical characteristics. Unsaponifiable matter was studied for its pharmacological profile.

1. Proximate Analysis and Qualitative Examination of Seeds.

Proximate analysis of seed kernels was carried out to lay down

* Department of Pharmaceutical Sciences,
Dr. Harisingh Gour Vishwavidyalaya, Sagar - 470003

Table - I

Proximate Analysis of Seed Kernels of *Sapindus trifoliatus* Linn.

Determination	% w/w
Moisture content	6.68
Total ash	4.00
Acid insoluble ash	0.298
Sulphated ash	45.88
Alcohol (95%) soluble extractive	9.77
Water soluble extractive	29.17

Table - II

Physico-chemical Characteristics of Seed Kernel Derived Fixed Oil of *Sapindus trifoliatus* Linn.

Characteristic	Value
Refractive index (20°C)	1.4675
Specific gravity (25°C)	0.8964
Acid value	1.54
Saponification value	191.90
Iodine value	56.96
Acetyl value	NIL
Unsaponifiable matter	0.60 w/w

Table - III

Physico-chemical Characteristics of Fatty Acids and Their Fractions from Seed Kernels of *Sapindus trifoliatus* Linn.

Characteristic	Mixed fatty acids	Saturated fatty acids	Unsaturated fatty acids
Neutralisation number	159.1	-	-
Mean molecular weight	352.6	-	-
Saponification value	231.3	223.8	234.7
Iodine balue	69.2	0.45	89.1

certain standards for the air dried drug and I.P. methods were followed for determining moisture content, total ash, acid- insoluble ash, sulphated ash, and alcohol-soluble and water-soluble extracts. Successive solvent extraction using solvents with increasing order of polarity was carried out, followed by qualitative tests for various plant constituents (Table-I).

2. Determination of Phsico-chemical Characteristics of Fixed oil and Fatty acids[6]

Fixed oil (48.8% w/w) extracted from dried and coarsely powdered seed kernels of *S. trifoliatus* with pet. ether (60-80°C) yielded 84.3% w/w mixed fatty acids. Following lead salt method, saturated (17.4% w/w) and unsaturated fatty acids (78.4% w/w) were separated[7]. Various physico-chemical characteristics of the above were observed and recorded (Table II &III.)

3. Co-TLC of Fatty Acids and Unsaponifiable matter

Saturated and unsaturated fatty acids obtained from seed kernel oil were converted into neutral methyl esters [7,8] and separated by thin layer chromatography[9,10]. The best separation was achieved by solvent system Pet. ether (60-80°) - ethyl acetate (95 : 5). Co-TLC with authentic samples of esters of stearic, palmitic and oleic acids was performed.

Unsaponifiable matter (0.6%) obtained after saponification of fixed oil was tested for the presence of sterols by (a) Hesse's test (b) Libermann's test and (c) Libermann-Burchard's test. Co-TLC[12] of isolated fractions of unsaponifiable matter using solvent system pet. ether (60-80°C) - ethyl acetate (95 : 5) with authentic sample of ß - sitosterol and subsequent determination of their melting points revealed one out of the two components to be ß-sitosterol (melting point - 137°C). Second component separated by column chromatography[13-15] using benzene as eluant could not be identified (Table - IV).

4. Pharmacological Studies of Unsaponifiable Matter

Unsaponifiable matter suspended in 5% gum acacia mucilage in a concentration of 20 mg/ml was used for the pharmacological studies and all the experiments were done in triplicate.

(A) Effect on Frog's Heart[15] (*in situ*)

A dose of 0.20 ml suspension amounting to 4.0 mg of unsaponifiable matter showed positive inotropic effect.

(B) Effect on Isolated *Rectus abdominus* Muscle of Frog[16]

Isolated piece of rectus abdominal muscle (2.5 cm) was suspended in Ringer's solution in inner organ bath (capacity - 40 ml) of student's isolated organ bath. Graded doses (2,4,6 and 8 mg/ml of bath conc.) of unsaponifiable matter exerted no effect against acetylcholine which showed characteristic contraction of muscle at bath concentration of 1 mg/ml.

(C) Effect on Isolated Ileum and Uterus of Rat[17]

Addition of graded doses (4 mg to 20 mg) of unsaponifiable matter in a organ bath (capacity - 40 ml) showed no effect on isolated ileum and uterus of virgin female albino rats.

(D) Effect on Metrazole Induced Convulsions in Rats[18]

Table - I

Proximate Analysis of Seed Kernels of *Sapindus trifoliatus* Linn.

Determination	% w/w
Moisture content	6.68
Total ash	4.00
Acid insoluble ash	0.298
Sulphated ash	45.88
Alcohol (95%) soluble extractive	9.77
Water soluble extractive	29.17

Table - II

Physico-chemical Characteristics of Seed Kernel Derived Fixed Oil of *Sapindus trifoliatus* Linn.

Characteristic	Value
Refractive index (20°C)	1.4675
Specific gravity (25°C)	0.8964
Acid value	1.54
Saponification value	191.90
Iodine value	56.96
Acetyl value	NIL
Unsaponifiable matter	0.60 w/w

Table - III

Physico-chemical Characteristics of Fatty Acids and Their Fractions from Seed Kernels of *Sapindus trifoliatus* Linn.

Characteristic	Mixed fatty acids	Saturated fatty acids	Unsaturated fatty acids
Neutralisation number	159.1	-	-
Mean molecular weight	352.6	-	-
Saponification value	231.3	223.8	234.7
Iodine balue	69.2	0.45	89.1

Table - IV
Co-TLC of Methylesters of Fatty Acids and Unsaponifiable Matter from Seed Kernel Oil of *Sapindus trifoliatus* Linn.

Solvent system	No. of spots	Rf values		Esters of fatty acids having same Rf value	Rf value of unsaponifiable matter	Sterols with same Rf value
		Esters of saturated fatty acids	Esters of unsaturated fatty acids			
Pet. ether - Ethyl acetate (95:5)	3	0.85 0.72 0.63.	- - -	- Stearic Palmitic	- - -	- - -
-Do-	2 -	- 0.58	0.43 -	Oleic -	-	- -
-Do-	2 -	- -	- -	-	0.82 0.23	ß-sitosterol -
Pet. ether - Ethyl acetate (90:10)	2	- -	- -	- -	0.95 0.29	ß-sitosterol -
Benzene	2 -	- -	- -	-	0.95 0.18	ß-sitosterol -

The method of Dandiya and Cullumbine was employed using six animals (50 - 70 gms) per group. Albino rats treated with 30 mg and 100 mg/kg dose of unsaponifiable matter, administered intraperitoneally, showed no protection against 70 mg/kg metrazole induced convulsions and no death was recorded within 24 hours of treatment.

(E) Effect on Electro-shock Induced Convulsions in Rats[19,20]

Two groups, each of four animals (70 -85 gms) were used for experiments. Albino rats when administered 30 mg/kg, ip, dose of unsaponifiable matter showed 25% protection against the electro-shock induced convulsions over control animals in terms of average time of extensor of hind limb.

DISCUSSION

Proximate analysis of seed kernels indicated the presence of inorganic matter in abundance. Qualitative examination of the various extracts of seed kernels indicated the presence of phytosterols and carbohydrates apart from some other common phyto-constituents. Three out of five fatty acids present in the fixed oil were identified to be palmitic, stearic and oleic acids by Co-TLC with authentic samples. One out of the two unsaponifiable components was identified to be ß-sitosterol.

The unsaponifiable matter showed positive inotropic effect on frog's heart and protection of moderate level against electro-shock induced convulsions in rats. It, however, did not show any acitivity on uterus.

REFERENCES

1. Nandkarni, K.M. and Chopra, R.N., "Indian Materia Medica", Vol. I, Popular Prakashan, Bombay. 1976, p, 1102.
2. Watt, G., " A Dictionary of the Economic Products of India ", Vol. VII, Part II , Periodical Experts, Delhi, 1972 - 46871.
3. Paranjpe, D.R. and Ayyar, P.R., *J.Indian Inst. Sci.*, 1929, *12A,* Part12 ,179-84, through *Chem.Abstr.*, 1930, *24,* 743.
4. Hilditch, T.P. and Williams, P.N., "The Chemical Constituents of Natural Fats", 4th Edn., Chapman and Hall Ltd. , London, 1964, p., 318.
5. Vijayalakshmi, B. and Rao, S.V., *Chem. Phys. Lipids*, 1972, *9(1),* 82-6, through *Chem.Abstr.*, 1972, 77, *46951d.*
6. "Pharmacopoeia of India", 2nd Edn., Ministry of Health and Family Welfare, Govt. of India, Controller of Publications , Delhi, 1966, p., 947.
7. Paech, K. and Tracey, M.V., "Modern Methods of Plant Analysis", Vol II, Springer - Verlag, Berlin, 1955, p. 347-49.
8. Hilditch, T.P. and Williams, P.N., "The Chemical Constituents of Natural Fats", 4th Edn., Chapman and Hall Ltd., London, 1964, p. 688.
9. K. Randerath, K., "Thin Layer Chromatography", 2nd Edn., Academic Press, New York, 1966, p. 18.
10. Marinetti, G.V., "Lipid Chromatographic Analysis", Vol., III, 2nd. Edn., Marcel Dekker Inc., New York, 1976. p. 753.

11. Stahl, E., "Thin Layer Chromatography", Academic Press Inc., New York, 1969 , p. 386.
12. *Ibid.*, p. 329.
13. Drach, J.C., "Remington's Pharmaceutical Sciences", 14th Edn., Editor-Chairman Arthur Osol , Mack Publishing Co., 1970, p. 675.
14. Beckett, A.H. and Stenlake, J.B. , "Practical Pharmaceutical Chemistry", Part II , 3rd Edn., The Athlone Press , London, 1976, p. 81.
15. Hilditch, T.P. and Williams, P.N., " The Chemical Comstituents of Natural Fats", 4th Edn., Chapman and Hall Ltd., London, 1964, p. 684-85.
16. Burn, J.H., "Practical Pharmacology", Blackwell Scientific Publication, Oxford, 1952, p. 30 & 15.
17 Turner, R.A.,"Screening Methods in Pharmacology", Academic Press, New York, 1965, p. 43 & 175.
18 Dandiya, P.C. and Cullumbine H., *J. Pharmacol. Exp. Therap.*, 1959, *125* , 353-54.
19 Andres Goth, M.D. , "Medicinal Pharmacology", 3rd Edn., Topan Co. Ltd., Tokyo, 1966, p. 268.
20 Turner, R.A., "Screening Methods in Pharmacology" , Academic Press, New York, 1965, p. 166.

REVIEW ARTICLE

REVIEW ON PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF TRIBULUS TERRESTRIS LINN

S.K. Gupta[2] R. Zafar[1] and D. Pathak[3]

(Received 22 February 1997)

INTRODUCTION : DESCRIPTION OF PLANT

Tribulus is a genus of ascending or prostrate herb, belonging to the family Zygophyllaceae, distributed in the tropics and warm - temperate regions of the world. Three species which are found in India are ***Tribulus terrestris, Tribulus Cistoides*** and ***Tribulus alatus***[1]. Among them T. terrestris L. is a trailing plant, common in sandy soil throughout India, upto 11000 ft. in Kashmir, Ceylon. The Plant is commonly known in Hindi: Chotagokhru, Punjabi: Bakhra; English: Calthrops.

It is a procumbent, ascending or suberect herb; stems and branches pilose, young parts villous. Leaves opposite, abruptly pinnate, one of each pair usually smaller than the other, sometimes wanting altogether; Stipules lanceolate, hairy; leaflets 3-6 pairs, oblong, mucronate, villous on both the surfaces; base rounded oblique; petioles minute, hairy. Flowers axillary or leaf opposed, yellow, solitary, hairy; pedicles filliform. Sepals lanceolate, acute, hairy. Petals oblongobloid, claw short, hairy; stamens 10, inserted on the base of the disk, alternately longer and shorter, the latter with a small gland outside, filaments filliform, naked ovary sessile, hirsute, 5-12 lobed and celled; Style short; stigmas 5-12; ovules superposed. Fruit globose with 5-hairy woodycocci, each with 2 spines. Seeds many in each coccus, with transverse partitions between them. Flowering and fruiting-hot season and rainy season.

Leaves are diuretic, tonic; increase the menstrual flow; cure gonorrhoea; a decoction is useful as a gargle for mouth trouble and painful gum and reduce inflammation.

The fruit is diuretic removes gravel from the urine and stone in the bladder. They are regarded as cooling, diuretic, tonic and aphrodisiac, and are used in painful micturition, calculous affections, urinary disorders and impotence. In some countries they are reputed tonic and astringent, used for coughs, scabies, anaemia and opthalmia.

The root is good stomachic and appetiser, diuretic and carminative.

The entire plant, but more particularly the fruits are used in medicines. It was given a good trial in Bright's disease with dropsy. The diuretic property of the drug is due to the presence of large quantities of nitrates present as well as the essential oil which occurs in the seeds[2].

PHYTOCHEMICAL STUDIES

Fruit contains an alkaloid in traces (0.001%); fixed oil 3.5% consisting mainly of unsaturated acids, essential oil in very small quantities, resins and fair amounts of nitrates[3]. Harman occurs in the herb and harmine in seeds. The plant contains saponins which on hydrolysis yield steroidal sapogenins. Kaempferol, Keempferol-3-glucoside, Kaempferol-3-

* For correspondence
[1] Dept. of Pharmacognosy & Phytochemistry, Faculty Pharmacy, Jamia Hamdard, Hamdard University, New Delhi - 110 062
[2,3] Dr. K.N. Modi Institute of Pharmaceutical Education and Research, Modinagar - 201 204 (U.P.)

rutinoside and a flavonoid tribuloside have been isolated from leaves and fruits[4].

Tribulus species cause the disease photosensitivity and geeldikkop in animals due to presence of an icterogenic principle in the plant was first studied by Henrici et al[5] and later by Brockmann, et al[6].

Diosgenin, Ruscogenin, Gitogenin and 25-D-Spirosta-3, 5-Diene obtained by hydrolysis of crude saponin isolated from *T. terrestris*[7].

Dried fruits of *T. terrestris L.* contains 5% of semidrying oil, peroxides, diastase, traces of glucosides, resins, protein and a large amount of inorganic matters[8]. Shah et al[9] reported the presence of vit. C in the whole plant (78.00-141.66 mg/100 gm).

Nath, et al[10] reported crude protein 12.06%, ether extract 2.61%; crude fibre 27.7%; nitrogen free extract 40.83%; total carbohydrates 68.61%; total ash 16.72%; calcium 4.21% and phosphorus 0.24%.

Three steroidal sapogenins, diosgenin, gitogenin and chlorgenin were isolated by Gheorghiu et al[11]. Out of 10 steroidal substances 3 saponins C, F & G (Fig. 1) were isolated from overground part of *T. Terrestris,* with the help of repeated column chromatography and thin-layer chromatography by Tomowa et al[12]. Saponin F proved to be a new product: tigogenin-3- diglucorhamnoside, named by them terrestroside F, the partial structure of which was determined on the basis of the hydrolytic products: aglycon tigogenin (identified by m.p., mixed m.p.; I.R. and mass spectrum; acetyl derivative) and an oligosaccharide part rhamnose: glucose (2:1). The saponins C and G proved to be a mixture of two tigogenin and diosgenin glycosides each. The mixture of aglycones was separated by column chromatography on silica gel containing silver nitrate and identified by the above mentioned indices as tigogenin and diosgenin. In the hydrolysates the sugars glucose and rhamnose were proved. A flavonoid was also isolated which was identified as astragaline (caempferol-3- glucoside).

Purushothaman, et al[13]. isolated two steroid sapogenins hecogenin (3 β-hydroxy-5α- spirostan 12-one) and neotigogenin 5 α: 22 25S-spirostan-3β-01) with the help of chromatography over silica gel from the chloroform extract of whole plant of *T. terrestris*, compound A, C_{27} H_{44} O_3 m.p. 199-201°, λmax 3500 cm^{-1}). Its monoacetate C_{27} H_{46} O_4, m.p. 170° (λmax 1725 and 1240 cm^{-1}). Compound B, C_{27} H_{42} O_4, m.p. 243°, Contains a hydroxyl group (3460 cm^{-1}) and a six membered ring ketone (1710 cm^{-1}) and its monoacetate, C_{27} H_{49} O_5, m.p. 240°. Hecogenin was also reported by Tomowa, et al[14].

Tomowa et al[15] established the structure of isolated glycoside from the over ground part of *T. terrestris L.* as furostanol bisglycoside protodioscin (Fig. - 2) which upon acid hydrolysis yield the spirostanol diosgenin, tigogenin, glucose and rhamnose. Mahato et al[16] analysed for diosgenin content from four samples of T. terrestris L. growing under different climate condition. The highest yield of diosgenin was 0.21% and the lowest yield was 0.06%. Other steroid constituents characterised were β-sitosterol, stigmasterol and neotigogenin.

Altogether 22 aminoacids were identified in the root nodules of *T. terrestris L.* and qualitatively analysed by Ather, et al[17]. Glutamic acid, Glutamine, Aspartic acid and Asparagine being the major amino acids. Other amino acids are cystine, cysteine, Tryptophan, serine, proline, Glycine, Alanine, Valine, Methionine, Leucine, Isoleucine, Tyrosine, Phenylalanine, γ-Amino butyric acid, Ornithine, Lysine, Histidine and Arginine.

Chakravarti, et al[18] isolated Diosgenin from the weeds of *T. terrestris L.* Seth, et al[30]., reported the Sodium, potassium, and Calcium contents in the fruits of *T. terrestris L.* Arti Duhan, et al[19]. reported a rich source of calcium in the leaves of *T. terrestric L.*

Afria[20] showed that young leaves possessed the maximum concentration of protein (92.5 mg./gm dry wt.) and most of the individual free amino acids[15], as compared with mature leaves and immature fruits.

Saleh et al[21]. detected 25 flavonoid glycosides in *T. terrestris L.* The glycosides belong to the common flavonols, kaempferol, quercetin and isorhamnetin with the 3-gentiobiosides as the major glycosides. Singh et al[22] isolated Diosgenin and Tigogenin from over ground part of *T. terrestris L.*

Prakash, et al[23]. confirmed 4 alkaloids harmine, harmaline, harman and tetrahydroharmine in the plant *T. terrestris.* Bourke et al[24]. extracted 5 compounds in the alkaloid mixture of *T. terrestris L.*, only 2 were present in large amount and identifiable as the structurally related beta-carboline indoleamines harmane and norharmane.

Zafar, R. et al[25]., isolated diosgenin, hecogenin, ruscogenin, spirosta-3,5-diene from flowers of *T. terrestris L.* Two compounds of cinnamic amide derivative named terrestriamide and 7-methylhydroindanone-1, were isolated from *T. terrestris L* for the first time[26].

PHARMACOLOGICAL SCREENING

Pharmacological study of *T. terrestris L* have been carried out by Bose et al[27]. The minor alkaloidal fraction did not affect the blood pressure of the dog, but depressed the frog heart in *situ.* It produced inhibition of acetyl choline induced contraction of isolated intestine of rats and also of frog rectus muscle and had moderate diuretic effect. The aqueous fraction induced mild hypotension, showed anti-acetylcholine like action on the rat intestine. The seeds of the *T. terrestris* was found to be toxic to the liver of rats[28]. No toxic symptoms were observed by Sastry[29]. Seth et al[30]. reported that water soluble extract of *T. terrestris L* had a potent stimulant effect on the isolated heart muscle in hypodynamic state. Chakraborty et al[31]. studied the various phamacological action and reported that an alcoholic extract of the plant produced a sharp vasodepression in anaesthetised dogs mediated through cholinergic mechanism. It also possessed some characteristic changes in C.N.S. and in carbohydrate metabolism. Prakash et al[23]. reported marked C.N.S. stimulant activity in adult albino mice in *T. terrestris L.* Bourke et al[32] reported locomotor disorders with the *T. terrestris L.* due to beta carboline alkaloid.

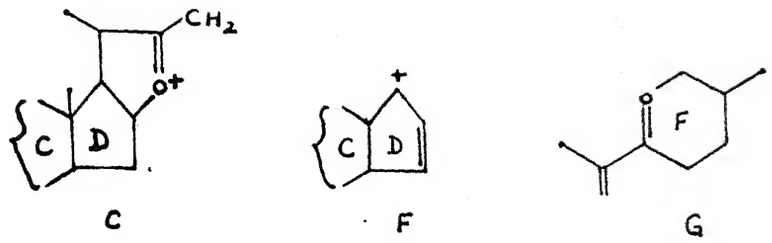
Bourke et al[24] administered harmane and norharmane from alkaloid extract of *T. terrestris L* to normal sheep and showed that both compounds were able to cause locomotor effects. Antiurolithiatic activity in the alcoholic extract of *T. terrestris* was studied by Anand, R et al[33]. Singh et al[34]., evaluated the diuretic action with minimal side effects of *T. terrestris L* on the albino rat.

Administration of the fractions of ethanolic extract of *T. terrestris* fruits by Anand R. et al[35] resulted in a varying degree of reduction in deposition of stone as compared to the untreated control animals.

Sangeeta et al[36]. observed the effect of an aqueous extract of *T. terrestris* on the metabolism of oxalate in male rats fed sodium glycolate, that lowering hyperoxaluria seemed to be mainly mediated through its inhibitory action on GAO and GAD, and its enhanced production of glyoxylate. Vijaya S. et al[37] examined *in vitro* that aqueous extract of *T. terrestris L.* inhibited Amylase and activated Lipase digestive enzyme.

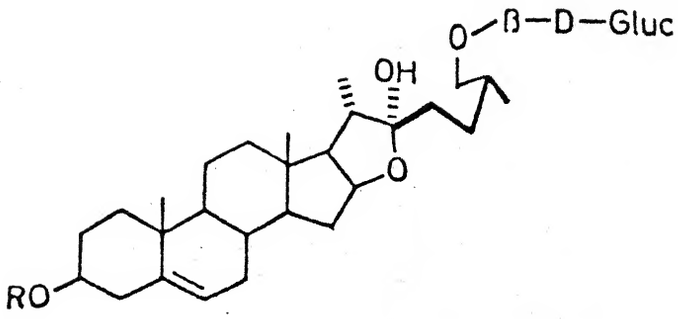
ANTI MICROBIAL SCREENING

George, et al[38] reported that alcoholic and aqueous extracts of plant or leaf are effective against *S. aureus* and *E. Coli* whereas the aqueous extract of seeds was only effective against *S. aureus.* Joshi *et al*[39] studied the antibacterial activity of 0.9% saline solution extract of fruit material, against the *S. aureus* and *E. Coli.* Dhar, *et al*[40] reported the antimicrobial activity of 50% ethanolic extract of the seed and



C F G

Fig. 1



R=Gluc: Rham (1:2)

Fig. 2

aerial parts of *T. terrestris against the B. Subtilis, S. Typhi, A. tumefaciens, E. Coli and M. tuberculosis.*

Singh, et al[41] reported that the ethanolic extract (95%) of *T. terrestris* (fruits) is completely active against *E. coli.*

Ikram, M. et al[42] studied the antimicrobial activity of ethanolic extract (95%) of *T. terrestris* (stem & leaf) against *B. Subtilis* by hole-plate diffusion method. Surinder Jit et al[43] reported maximum activity in ether and 50% ethanolic (1:1) extract of *T. terrestris* shoot against *S. aureus.*

Twaij, H.A.A. et al[44] found the molluscicidal activity at 50-100 ppm and most toxic at 100-200 ppm concentration against *Bulinus truncatus* in the aqueous extract of *T. terrestris L.*

REFERENCES

1. Anonymous, **"The Wealth of India"**, Raw Materials, Vol. X, 1982, C.S.I.R., New Delhi, p. 283.
2. Kirtikar, K.R. and Basu, B.D., **Indian Medicinal Plants,** 1987, I: 420 - 24.
3. Nadkarni, K.M. **Indian Materia Medica**, 1993, I: 1129 - 32.
4. Verma, Rajani, **J. Nat. Integ. Med. ASS.** 1980, 22(9): 234- 36.
5. Henrici and ondersetepoort; **J. Vet. Sci. Animal Ind.**, 1938, 10: 367, S. African J. Sci., 1946, 43: 195.
6. Brockmann and Forsch V., **Fortschr.**, 1943, 19, 299.
7. De, Kock, W.T. and Enslin, P.R., **J.S. African Chem. Inst.** 1958, 11, 33 - 6.
8. Ghatak, N., **Bull. Acad. Sci. United. Provinces Agra and Oudh. India,** 1933, 2: 163 - 70.
9. Shah, F.H. and Bhatty M.K., **Pakist, J. Sci, Res.**, 1962, 14(I): 4 - 7.
10. Nath, K. and Malik, N.S., **Indian J. Anim. Sci.** 1970, 40(4) 434 - 437.
11. Gheorghiu A and Tonescu-Metiu, **E. Stud. Cercet. Biochim,** 1968, 11(3): 269.
12. Tomowa, M.P., Panowa, D. and Wulfson, N.S. **Planta Med.** 1974, 25(3), 231-237.
13. Purushthaman, K.K., Chandrasekharan S. and

Balakrishna, K. **Jour. Res. Ind. Med. Yoga & Homoeo.**, 1976, 11(I): 121 - 125.

14. Tomowa, M.P., Botschewa, D.M., Zaikin, W.G. and Wulfson, N.S., **Planta Med.**, 1977, 32(3), 223 - 224.

15. Tomowa, M.P., Gjulemetowa, R., **Planta Med.**, 1978, 34(2): 188 - 191.

16. Mahato, S.B., Sahu, N.P. and Pal, B.C., **J. Inst. Chemists (India).**, 1978, 50(I): 49 - 50.

17. Athar, M. and Mahmood A., **Pak J. Bot.**, 1980, 12(I), 91- 96.

18. Chakravarti, R.N., **Chem. Abstr (Biochem)**, 1980, 92(6), 329, **abstr.**, 91186d.

19. Duhan, A., Chauhan B.M. and Punia, D. **Plant Foods Hum. Nut.**, 1992, 42(3): 193 - 200.

20. Africa, B.S., **Geobios.**, 1980, 7(3): 121 - 123.

21. Saleh, N.A.M., Ahmed, A.A. and Abdalla, M.F., **Phytochemistry.**, 1982, 21(8): 1995-2000.

22. Singh, S.B. and Thakur, R.S., **J. Sci. Industr. Res.**, 1983, 42(6): 319 - 334.

23. Prakash, D. Singh, P.N. and Wahi, S.P. **Indian Drugs.**, 1985, 22(6): 332-3.

24. Bourke, CA, Carrigan M.J. and Dixon, R.J., **Aust. Vet. J.**, 1992, 69(7), 163-4.

25. Zafar, R. and Aeri, V., **Fitoterapia.**, 1992, 63(1): 90.

26. Ren, Y.J. and Chen, H.S. **Acta. Pharma. Sinica.**, 1994, 29(3), 204-206.

27. Bose, B.C. Saifi, A.Q., Vijay Valglya, R. and Bhatnagar, J.N., **Indian J. Med. Sci.**, 1963, 17, 291-3.

28. Toxicity of Gokhru seeds., **T. terrestris L. Indian For.**, 1963, 89(12): 778.

29. Sastry, M.S., **Agric. Res. New Delhi, 1964, 4(I): 54.**

30. Seth, S.D. and Jagadesh G., **Indian J. Med. Res.**, 1976, 64(12): 1821 - 25.

31. Chakraborty, B. and Neogi, N.C., **Indian J. Pharm. Sci.**, 1978, 40(2): 50-52.

32. Bourke. CA, Carrigan, MJ. and Dixon, RJ., **Aust. Vet. J.**, 1990, 67(7): 248 : 251.

33. Anand R., Patnaik G.K., Kulshrestha, D.K. and Dhawan, B.N., **Indian J. Pharmacol.**, 1989, 21(I): 74.

34. Singh, R.G., Singh, R.P., Usha, Shukla K.P. and Singh, P., **Jour Res. Edu. Ind. Med.**, 1991, 10(I): 19-21.

35. Anand, R. Patnaik G.K., Kulshreshtha, D.K., & Dhawan B.N., **Indian J. Exp. Biol.**, 1994, 32(8): 548 - 52.

36. Sangeeta, D., Sidhu, H., Thind, S.K. and Nath, R.J., **Ethnopharmacol.**, 1994, 44(2): 61-66.

37. Vijaya, S. and Vasudevan, T.N., **Indian Drugs.**, 1994, 31(5): 215-17.

38. George, Mariam (Miss)., **J. Sci. Industr. Res.**, 1947, 6B(3): 42-46.

39. Joshi, C.G. and Magar, N.G., **J. Sci. Industr. Res.**, 1952, 11B(6): 261.

40. Dhar, M.L., Dhar, M.M., Dhawan, B.N., Malhotra, B.N. and Roy. C., **Indian J. Exp. Biol.**, 1968, 6(4): 232-47.

41. Singh, R.H. Khosa, R.L. and Upadhaya B.B., **Jour. Res. Indian. Med.**, 1974, 9(2): 65 - 66.

42. Ikram, M. and Haq. Inamul. **Fitoterapia.**, 1980, 51(5): 231-35.

43. Surinder Jit., Shekhawat. S., Grover Sharad and Nag., T.N., **Acta Botanica Indica.**, 1986, 14(I): 45-47.

44. Twaij, H.A.A., Mahmoud, S.N. and Khalid, R.M., **Fitoterapia.**, 1989, 60(3): 267-8.

Asian Journal of Chemistry Vol. 17, No. 1 (2005), 33–36

MICRO-REVIEW

Review on Phytochemical and Pharmacological Aspects of *Cichorium intybus* Linn.

S.K. GUPTA* and S.H. ANSARI†
Department of Pharmaceutical Chemistry
Dr. K.N. Modi Institute of Pharmaceutical Education and Research,
Modinagar-201 201, India

Cichorium intybus Linn. (Compositae) is an important medicinal plant which finds use in Ayurveda and Unani systems of medicine, especially in inflammations. It is useful in thirst, headache, ophthalmia, throat inflammation, enlargement of the spleen, fever, vomiting and diarrhoea etc. An attempt has been made to review the phytochemical and pharmacological work done on *Cichorium intybus* Linn.

Key Words: Review, *Cichorium intybus* Linn., Phytochemical and pharmacological properties.

INTRODUCTION

Cichorium is a genus of thirteen species belonging to the family Compositae. Two species, *viz.*, *C. endivia* and *C. intybus*, are of common occurrence in N.W. India up to 6,000 ft., Waziristan, Baluchistan, W. Asia and Europe. *C. intybus* Linn. has been described to be of great medicinal value. *C. intybus* is a perennial herb, 1–3 ft. high, with fleshy tap root up to $2^1/_2$ ft. in length. The plant is commonly known in Hindi: Kasni; Punjabi: Hand; English: Chicory[1].

Morphology

An erect, usually rough and more or less glandular, perennial herb; stems 0.3–0.9 m, angled or grooved; branches tough, rigid, spreading; radical and lower leaves 7.5–15 cm, pinnatifid lobes toothed, pointing downwards; upper leaves alternate, small, entire, heads ligulate, 2.5–3.8 cm diam.; flowers bright blue; pappus of 1 or 2 series of short, blunt erect scales; ligules very long, spreading, 5-toothed; style-arms long; achenes smooth, angled, crowned with the ring of pappus scales.

The plant is a good tonic, cooling, useful in thirst, headache, ophthalmia, throat inflammation, enlargement of the spleen, fever, vomiting and diarrhoea. The root is stomachic and diuretic; enriches and purifies the blood; lessens inflammation and pain in the joints. The seeds are tonic to the brain, alexiteric, appetiser; useful in ophthalmia, biliousness, lumbago, troubles of the spleen and asthma. The leaves are applied topically to lessen pain in the joints and have also hypoglycaemic effect. The flowers are used in liver disorders.

†Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India.

Photochemical Investigations

Seeds contain a bland oil, 4.5%; fresh roots contain moisture, 77%; gummy matter, 7.5%; glucose, 1.1%; bitter extractive, 4.0%; fat, 0.6%; cellulose, inulin and fibre, 9.0% and ash, 0.8%. The ash of the roots and also of the leaves is rich in potash. Betaine and choline are also present in small concentrations. Flowers contain a colourless crystalline glucoside; cichoriin, bitter substances lactucin and intybin[1–4].

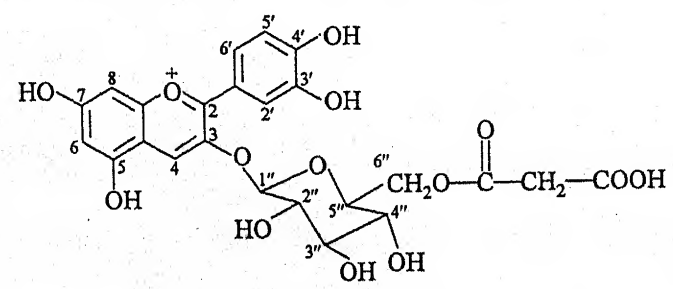
Barakat *et al.*[5] reported average of ferric iron content 3.14 mg% and cupric copper content 0.17 mg% by ascorbimetry. Balbaa *et al.*[6] reported the presence of flavonoids, catechol tannins, glycosides, carbohydrates, unsaturated sterols, triterpenoids and the absence of alkaloids, oxidase enzyme and saponins in the roots of each of eight varieties of *C. intybus* L.

Wight *et al.*[7] determined reducing sugars, sucrose and inulin content in roots of *C. intybus* L. Bridle *et al.*[8] identified that major anthocyanin is cyanidin 3-O–β-(6-O-malonyl)-D-glucopyranoside **(1)** by fast atom bombardment mass spectrometry and NMR spectroscopy in red leaves of *C. intybus* L.

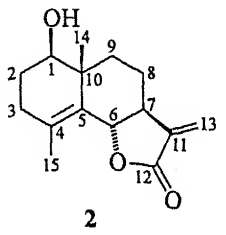
Takeda *et al.*[9] identified a pigment, delphinidin 3-(6-malonylglucoside)-5-malonylglucoside, in blue flowers of *C. intybus* L. Saleem *et al.*[10] examined the seed oil from *C. intybus* for its physico-chemical values and fatty acid composition by gas chromatography. Grayer *et al.*[11] reported an antifungal compound, cichoralexin, in leaves of *C. intybus L.*

Park *et al.*[12] isolatated two known eudesmanolides, magnolialide and artesin from the roots of *C. intybus* and their structures were identified as magnolialide [1β-hydroxyeudesma-4,13-dien-6,12-olide **(2)**] and its 11β-13-dihydro derivative **(3)** respectively. The known eudesmanolide magnolialide and the known guainolide ixerisoside-D reported from *C. intybus*; along with the previously known sesquiterpene lactones, have also been isoated and identified by Kisiel *et al.*[13]

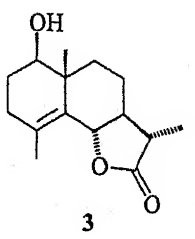
Four anthocyanin pigments were isolated from flowers of *C. intybus* and identified as delphinidin 3,5-di-O-(6-O-melonyl-β-D-glucoside) **(1)** and delphinidin 3-O-(6-O-malonyl-β-D-glucoside)-5-O-β-D-glucoside **(2)** and the known compounds were delphinidin 3-O-β-D-glucoside-5-O-(6-O-malonyl-β-D-glucoside) **3.** and delphinidin 3,5-di-O-β-D glucoside. **(4)** as shown in Fig. 1. in addition, 3-O-*p*-coumaroyl quinic acid has been identified by Norbeck *et al.*[14]



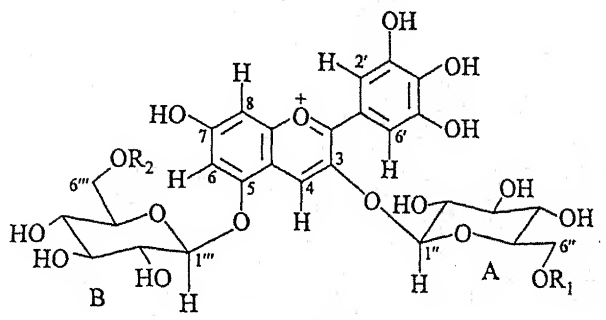
1



2



3



4

	R_1	R_2
1	malonyl	malonyl
2	malonyl	H
3	H	malonyl
4	H	H

Pharmacological Screening

Balbaa *et al.*[5] observed quinidine like action on isolated toads's heart in roots of each of eight varieties of *C. intybuys* L. Prakash *et al.*[15] observed 84% resorptive activity at a dose of 200 mg/kg body weight in 50% ethanolic extract of *C. intybus*. L.

Panday[16] observed bradycardia in normal and hypodynamic heart of frog and a fall in B.P. with a corresponding increase in respiratory rates in dog treated with alcoholic extract of seeds of *C. intybus* L. Handa *et al.*[17] reported cholagogue activity in alcoholic extract of the *C. intybus* L.

A significant decrease in the triglyceride level of liver, plasma and heart coupled with decreased cholesterol level in plasma was observed in rats, fed with high level of saturated fat supplemented with 5% roots of *C. intybus* L. as compared to high fat fed group, by Kaur *et al.*[18]. Misra, *et al.*[19] found antimalarial activity against erythrocytic stages of *Plasmodium berghei* only *in vitro* in alcoholic exract of seeds of *C. intybus* L.

Gadgoli *et al.*[20] found hepatoprotective activity against carbon tetrachloride and paracetamol induced toxicity in rats, treated each with chloroform, methanol and water extract of seeds of *Cichorium intybus* L.

Zafar *et al.*[21] reported better antihepatotoxic effect against carbon tetrachloride induced heptocellular damage in albino rats, treated with root callus extract as compared to the natural root extract of *Cichorium intybus* L.

Antimicrobial Activity

Abou-Jawdah *et al.*[22] found antimycotic activity against phytopathogenic fungi in pertroleum ether extract of *C. intybus* L.

REFERENCES

1. Anonymous, The Wealth of India, Raw Materials, PID, New Delhi, pp. 556–561 (1992).
2. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Books Distributors, Dehradun, pp. 1433–35 (1988).
3. S.Y. El-Gammal, *Hamdard*, **25**, 37 (1982).
4. K.M. Nadkarni, Indian Materia Medica, Popular Prakshan, Bombay, pp. 313–4 (1993).
5. M.Z. Barakat, M. Bossiouni and M. El-Wakil, *J. Sci. Food Agric.*, **23**, 685 (1972).
6. S.I. Balbaa, A.Y. Zaki, S.M. Abdel-Wahab, E.S.M. El-Denshary and M. Motazz-Bellah, *Planta Med.*, **24**, 133 (1973).
7. A.W. Wight and P.J. Van Niekerk, *J. Agric. Food Chem.*, **31**, 282 (1983).
8. P. Bridle, R.S.T. Loeffler, C.F. Timberlake and R. Self, *Phytochemistry*, **23**, 2968 (1984).
9. K. Takeda, J.B. Harborne and R. Self, *Phytochemistry*, **25**, 1337 (1986).
10. M. Saleem, M. Ahmad, M. Amin, S.A. Khan and M.K. Bhatty, *Pakistan J. Sci. Ind. Res.*, **30**, 245 (1987).
11. R.J. Grayer and J.B. Harborne, *Phytochemistry*, **37**, 19 (1994).
12. H.J. Park, S.H. Kwon, K.Q. Yoo, W.T. Jung, K.T. Lee, J.I. Kim and Y.N. Han, *Nat. Prod. Sci.*, **6**, 86 (2000).
13. W. Kisiel and K. Zielinska, *Phytochemistry*, **57**, 523 (2001).
14. R. Norbeck, K. Nielsen and T. Kondo, *Phytochemistry*, **60**, 357 (2002).
15. A.O. Prakash and R. Mathur, *Indian J. Exp. Biol.*, **14**, 623 (1976).
16. V.N. Pandey, *Bull., Medico-Ethno-bot. Res.*, **6**, 107 (1985).
17. S.S. Handa, A. Sharma and K.K. Chakarborti., *Fitoterapia*, **57**, 307 (1986).
18. N. Kaur, A.K. Gupta and B.Saijpaul, *J. Ethnopharmacol.*, **23**, 343 (1988).
19. P. Misra, N.L. Pal, P.Y. Guru, J.C. Katiyar and J.S. Tandon, *Int. J. Pharmacog.*, **29**, 19 (1991).
20. C.H. Gadgoli and S.H. Mishra, *Indian J. Pharm. Sci.*, **56**, 157 (1994).
21. R. Zafar and S.M. Ali, *J. Ethnopharmacol.*, **63**, 227–231 (1998).
22. Y. Abou-Jawdah, H. Sobh and A. Solameh, *J. Agric. Food Chem.*, **50**, 3208 (2002).

(*Received*: 27 May 2004; *Accepted*: 6 July 2004) AJC-3489

Asian Journal of Chemistry Vol. 17, No. 1 (2005), 37–39

MICRO-REVIEW

Review on Phytochemical and Pharmacological Aspects of *Dolichos biflorus* Linn

S.K. GUPTA* and P.K. SHARMA†
Department of Pharmaceutical Chemistry
Dr. K.N. Modi Institute of Pharmaceutical Education and Research,
Modinagar-201 201, India

Dolichos biflorus Linn. (Leguminosae) is an important medicinal plant which finds uses in Ayurveda and Unani systems of medicine especially for removing kidney stones. It has diuretic and emmenagogue effects. An attempt has been made to review the phytochemical, pharmacological and antimicrobial works done on this plant.

Key Words: Review, *Dolichos biflorus* Linn, Phytochemical and pharmacological properties.

INTRODUCTION

Dolichos is a well known and widespread genus of twining herbs of the family Leguminosae (Papillionaceae) occurring mainly in the tropical countries. It occurs all over India up to an altitude of 5000 ft. About 14 species occur in India, of which *D. biflorus* (Horse Gram), *D. lablab* (Bean), *D. catijang* (cow gram), *D. pruriens* (Cow hedge) and *D. soja* (Soya bean) are extensively cultivated and its seeds are used as food and leaves and stem as fodder. The seeds have been used in the indigenous system of medicine for a long time as astringent, anthelmintic, nerve tonic, diuretic, aphrodisiac and antipyretic etc. The plant is commonly known in *Hindi*: Kulthi; *Sanskrit*: Kulastha; *Bengali*: Kulti, Kurtikalai; *Marathi*: Kulith, Kulthi; *Gujarati*: Kulti; *Malayalam*: Kullu, Kollu; *Telugu*: Vlavalu; *Tamil*: Kollu.

Morphology

Stems: Very wide climbing slender, slightly pubescent, oblong blunt, subglabrescent leaflets on a petiole, lateral ones very unequal sided, stipullae minute and linear.

Flowers: 1–3 on very short pedicels in the axils of the leaves. Calyx slightly downy with upper teeth quite connate, the side lanceolate and the lowest one linear. Corolla yellow.

†Institute of Pharmacy, Bundelkhand University, Jhansi, India

Pods: Linear, subsessile, nearly straight, glabrous, 6–8 seeded, tipped with a persistant style.

Phytochemical Investigations

The seed has moisture, 11.8%; crude protein, 22.0%; fat, 0.5%; minerals, 3.1%; fibre, 5.3%; carbohydrates, 57.3%; calcium, 0.28%; phosphorus, 0.39%; iron, 0.0076%; nicotinic acid, 0.0015%; carotene, 119 IU/100 g, arginine 6.0–7.1%, tyrosine 6.68% and lysine 7.64%. Other important constituents of *D. biflorus* are strepogenin, β-sitosterol, bulbiformin, linoleic acid (in the seeds oil, 30–60%), polyphenols, oxalates (40% soluble) and crude fibre (5.3%)[1–3]. Pant *et al.*[4] found moisture 10.58%; ash, 3.86%; fat, 2.26% and crude protein, 21.35% in seeds. Mahadevappa *et al.*[5] reported palmitic acid, linoleic acid, oleic acid and linolenic acid in seed oil of *D. biflorus* L.

Mary *et al.*[6] isolated unusual enzyme allantoinase from germinated seeds of *D. biflorus* L. Seeds of *D. biflorus* L. contain total lipids 1.7–2.2%, neutral lipids 46–52% of total lipids, glycolipids 10–12% and pospholipids 35–40% of total lipids. Its amino acid composition is aspartic acid, lysine, phenyl-alanine, glycine, threonine, alanine, tyrosine, valine, glutamic acid, leucine, proline, serine and tryptophan. Seeds are rich source of ribonuclease. The glycosidases β-H-acetyl glucosamanidase, α- and β-galactosidases, α-mannosidase and β-glucosidase have been isolated and purified[7]. Singh, *et al.*[8] isolated phytohemagglutinin from the seeds and characterized by Kuehnemund *et al.*[9] as a glycoprotein of molecular weight about 130000 with amino acids and carbohydrates (0.5% galactose, 0.2% mannose, rhamnose and fructose).

Keen *et al.*[10] isolated genistein, 2′-hydroxy genistein, dalbergioidin, kievitone, phaseollidin and isoferrerin isoflavones after inoculation by some non-pathogenic bacteria, along with coumestrol and psoralidin form the leaves and stems of *D. biflorus* L. Ingham *et al.*[11] isolated two minor isoflavonoids dolichin A and B from the bacteria treated leaves of *D. biflorus* L.

Mitra *et al.*[12] isolated 5-hydroxy-7,3′,4′-trimethoxy-8-methylisoflavone and 5-neohesperidoside isoflavone from the ethanolic extract of seeds of *D. biflorus* L. Akihisa, *et al.*[13] isolated and identified fourteen triterpene alcohols and one 3-oxosteroid: stigmastenone [(24R)-stigmast-4-en-3-one] from seeds of *D. biflorus* L.

Dubey *et al.*[14] identified D-glucose, D-galactose, L-rhamnose, D-arabinose and L-ascorbic acid along with amino acids, *viz.*, glycine, alanine, cysteine, serine and aspartic acid from seeds of *D. biflorus* L.

Pharmacological Screening

The seeds are diuretic; emmenagogue; increase appetite; remove stone from kidney; cure hiccough, eye troubles, piles, enlargement of the spleen, pain in the liver; improve the complexion; cause biliousness. The decoction is used in leucorrhoea and menstrual derangements.[15] Kamboj *et al.*[16] reported that no anti-implantation activity at a dose of 200 mg/kg on days 1–7 post-coitum in rats for the petroleum ether, alcohol and aqueous extracts of seeds of *D. biflorus* L.

Laskar *et al.*[17] found antihepatotoxic activity in seeds of *D. biflorus* L. against paracetamol intoxicated rats at a dose of 10 mg/kg.

Antimicrobial Screening

Basak *et al.*[18] found antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, proteus vulgaris* and *Bacillus subtilis* in methanolic extract of seeds of *D. biflorus* L.

REFERENCES

1. Anonymous, The Wealth of India, Raw Materials, PID, New Delhi, pp. 161–162, (1952).
2. K.M. Nadkarni, Indian Materia Medica, Popular Prakashan, Bombay, pp. 458–459, (1993).
3. R. Zafar, Medicinal Plants of India, CBS Publishers & Distributors, Delhi, p. 70 (1994).
4. R. Pant and D.R.P. Tulsiani, *J. Agric. Food Chem.*, **17**, 361 (1969).
5. V.G. Mahadevappa and P.L. Raina, *J. Agric. Food Chem.*, **26**, 124 (1978).
6. A. Mary, and K.S. Sastry, *Phytochemistry*, **17**, 397 (1978).
7. M. Ikram, *Hamdard*, **24**, 102 (1981).
8. G. Singh and G.W.G. Bird, *J. Sci. Ind. Res.*, **15C**, 182 (1956).
9. O. Kuehnemund, W. Kochler, O. Prokop and Z. Hoppeseylers, *Physiol. Chem.*, **349**, 1434 (1968).
10. N.T. Keen, J.L. Ingham and Z.C. Naturforsch, *Biosci.*, **35**, 923 (1980).
11. J.L. Ingham, N.T. Keen, K.R. Markham and L.J. Mulheirn, *Phytochemistry*, **20**, 807 (1981).
12. J. Mitra, A. Das and T. Joshi, *Phytochemistry*, **22**, 1063 (1983).
13. T. Akihisa, Y. Kimura, K. Roy, P. Ghosh, S. Thakur and T. Tamura, *Phytochemistry*, **35**, 1309 (1994).
14. K.S. Dubey and A.H. Ansari, *Asian J. Chem.*, **10**, 209 (1998).
15. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, International Book Distributors, Dehradun, pp. 805-807 (1988).
16. V.P. Kamboj and B.N. Dhawan, *J. Ethnopharmacol.*, **6**, 191 (1982).
17. S. Laskar, U.K. Bhattacharyya, A. Sinhababu and B.K. Baskar, *Fitoterapia*, **69**, 401 (1998).
18. B. Basak, S.M. Ghosh, U. Bhattacharya, S. Laskar, A. Bandopadhyay and S.K. Sen, *Hindustan Antibiotics Bull.*, **34**, 100 (1992).

(*Received*: 27 May 2004; *Accepted:* 6 July 2004) AJC-3490

Asian Journal of Chemistry Vol. 17, No. 1 (2005), 40–44

Time-dependent Migration of Elements from Plastic-packaging Material into Food

MOHAMMAD W. KADI
Department of Chemistry, King Abdul Aziz University
P.O. Box 80203, Jeddah 21589, Saudi Arabia
E-mail: mkadi@kaau.edu.sa

Packaging is important for the food industry. Migration of elements from packaging material into food has attracted interest because of possible contamination of the food. In this paper behavior of migration with time was studied. Ca, Mg, Zn and K were used as examples for migration into food simulant. The study shows that polymer material, time of contact and type of migrating element affect the migration process.

Key Words: Packaging, Migration, Food.

INTRODUCTION

Polymer based packages have grown in popularity and are used all over the world for various applications. Packaging is essential to the food industry. It is used in a variety of applications, from simple containment of food to designed packages to prolong the shelf-life of the product. Along with the main polymer material, additives are often used to improve the performance of the package and to make it useful for specific applications. Examples of additives are coloring agents, plasticizers, stabilizers, anti-static agents, lubricants and antioxidants.

When the package comes into contact with food, two-way mass transfer takes place. Mass is transferred from the polymer into food; on the other hand mass is transferred from food into the polymer. These two processes are related and could be affected by many physical and chemical factors.

Recently, numerous studies showed that packaging might pose a problem, through migration of contaminants from the packaging material into food. Modeling studies try to simulate and predict the nature of the migration process. In these studies packaging material is subjected to extreme conditions and possible contamination is studied[1]. Other studies concentrate on qualitative and quantitative aspects of the migrants[2,3]. Extracting possible migrants from packaging material is another way to study food contamination[4–6]. In their study Castle *et al.*[4,5] extracted certain migrants from paperboard packaging material. In a study by Begley *et al.*[6], nylon packaging material was dissolved in organic solvents and possible migrants were studied.

Along with the main packaging material itself (the polymer), additives to the polymer used to improve the quality of the package can be a source of

Review on Phytochemical and Pharmacological Aspects of *Cassia tora* Linn.

R. Zafar and S.K. Gupta*
Dept. of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-62. *Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar-201204.

Abstract

***Cassia tora* Linn. (Leguminosae) is a common herbaceous annual plant occuring as weed throughout India, having a great reputation to be useful in all kinds of skin diseases; for ringworm and itch etc. It has laxative, diuretic, antihepatotoxic effects. In this article, an attempt has been made to review the phytochemical, pharmacological and antimicrobial work done on this plant.**

Keywords : *Cassia tora* Linn., Leguminosae, Laxative, Diuretic, Antihepatotoxic

Cassia tora Linn. is a common herbaceous plant belonging to the family Leguminosae, occurring as weed , throughout India, Sri Lanka and the tropics. The plant has a number of vernacular names e.g. Hindi:- Chakunda, Sanskrit:- Dadamardana, Gujrati:- Kovaraya, Marathi:- Takla, Takli, Tamil:- Tagarai etc (1).

Morphology

Leaves are 7.5-10 cm long, rachis grooved, more or less pubescent with a conical gland between each of the 2 lowest pairs of leaflets, stipules 1.3-2 cm long linear subulate and caducous. Leaflets 3 pairs, opposite, 2.5-4.5 by 1.3-2.5 cm (the lowest pair is smallest), obovate-oblong, glaucous, membranous, glabrous or more or less pubescent, base somewhat oblique, usually rounded, main nerves 8-10 pair; petiolules 2.5 mm long and pubescent.

Flower usually in subsessile pairs in the axils of the leaves, the upper crowded, common peduncle in fruit not exceeding 4 cm long; pedicels in fruit rarely exceeding 8 mm long. Calyx glabrous, divided to the base; segments 5 mm long, ovate, acute, spreading, Petals 5, pale yellow, subsequal, 8 by 2.5 mm, oblong, obtuse, spreading, the upper petal 2-lobed, the others entire. Stamens 10, the 3 upper reduced to minute staminodes, the remaining 7 perfect and subsequal.

Pods 12.5-20 cm by 4.5 mm, subtetragonous, much curved when young, obliquely septate, not reticulate and sutures are very broad. Seeds 25-30, rhombohedral, with the long axis in the direction of the pod

Both the leaves and seeds are laxative and useful in skin diseases. The leaves are used as an antiperiodic, aperient and anthelmintic. The root is considered bitter tonic and stomachic. The seeds are used as aperient, antiasthenic and diuretic agents and also to improve visual acuity in Chinese medicine. In Korea, the hot aqueous extract of the seeds is taken orally for the protection of the liver (2). The seed contain a glycoside and fixed oils (5%) (3).

Phytochemical Investigations

Naryana *et al* (4) isolated three crystalline substances which belong to the group of xanthones from the seed of *C. torra* L.. Ghosal *et al* (5) isolated water soluble alkaloid trigonelline from leaves, stem and pods of *C. torra* L.. Tiwari, *et al* (6) isolated anthraquinone pigment 1,3,5-trihydroxy- 6,7-dimethoxy- 2- methyl anthraquinone; Leucopelargonidin- 3- α-L-rhamnopyranoside and βV-sitosterol from ethanolic extract of the roots of *C. torra* L..

Acharya *et al* (7) isolated chrysophanic acid –9-anthrone from benzene extract of seeds of Cassia torra L.. Raghunathan *et al* (8) isolated two glycoside rubrofusarin-6-β-gentiobioside and a new anthraquinone chrysophanol-1-β- gentiobioside from etanol extract of seeds of *C. torra* L.. Niranjan *et al* (9) isolated proteins from seeds of *C. torra* L.. Singh *et al* (10) identified, glucose, galactose, xylose and raffinose from defatted seeds of *C. torra* Linn using T.L.C. method. Further Katoch et al (11) reported that immature seeds of the plan had higher level of crude protein (26.60 %) than the mature seeds (22.62%) . Chakrabarty *et al* (12,13) reported 3,5,8,3', 4',5' –hexahydroxyflavone, hydroxy coumarin , aurapterol, euphol, basseol, emodin, rhein, palmitic acid, isostearic acid, behenic acid, ethyl arachidate and β-sitosterol in stem bark and ethyl arachidate, β-sitosterol, behenic acid, palmitic acid, marginic acid, euphol and 3,5,8,3'4'5'-hexahydroxyflavone in leaves of *Cassia torra* linn. Upadhyaya *et al* (14) isolated monohydroxy anthraquinone, chrysoobtusin, free aminoacids, α-aspartic acid, cystine, canavine, β-cynoalanine, α-glutamic acid, γ-hydroxyarginine, l-proline, l-serine, tyrosine and valine, kaempferol, leucocanthocyanidins, chrysophanol, physcion, emodin, myricyl alcohol, quercetin, leucopelargonidin, stigmasterol and β-sitosterol from root and leaves of *C. torra* Linn.

Miralles *et al* (15) obtained 5.4 % oil from seeds. They reported unsaturated fatty acids, 68.2 % with a prominence of linoleic 44.6 %, oleic acids 21.6 % with amall amounts of malvalic and sterculic acids and 15 sterols.

Wong *et al* (16,17) isolated three new anthraquinone glycosides. Further two new naphtho- glycosides together with cassiaside and a gentiobioside were isolated from the methanolic extract of seeds of *Cassia tora* Linn.

Choi et al (18) isolated new naphthalene glycoside (cassitoroside) from seeds of *Cassia tora* L..

A new naphthopyrone glycoside was isolated from the roasted seeds of Cassia tora L. along with isorubrofusarin gentiobioside, alatemin and adenosine (19).

Pharmacological Screening

The crude extract of the leaves of *Cassia tora* L. was found to be lethal when a dose of 200 mg/ Kg , 100 mg/Kg and 20 mg/ Kg was given orally, intraperitoneally and intravenously respectively in mice. Death of mice occurred within 20 hours after administration of the drug (20).

The two anthraquinone glycosides exhibited a weak protective effect on primary cultured hepatocytes against carbon tetrachloride toxicity (16). The naphtho-γ-pyrone glycosides were found to have significant hepato-protective effects against galactosamine damage (17).

Methanolic extract of the leaves of *Cassia tora* at a dose of 400 mg/ Kg orally exhibited significant protective effect in rats in carbon tetrachloride induced hepatotoxicity (21, 22). The methanolic extract of leaves (400mg/ Kg) of this drug exhibited significant anti-inflammatory activity against carrageenin-, histamine –, serotonin and dextran – induced rat hind paw oedema (23). Leaves (200 mg/ 100 g body weight) exhibited maximum antifertility activity to be related to estrogenic activity in female rats (24).

Antimicrobial Activity

Acharya et al (7) found fungicidal activity in chrysophanic acid- 9- anthrone, isolated from ethanolic extract of seed. Singh et al (25) reported that the ethanolic extract (95%) of seeds had slight antibacterial activities against *E. coli in vitro*

Saxena et al (26) reported that petroleum ether and ethanolic extracts of seed were inactive against pathogenic fungi (*Aspergillus fuminatus*, *Trichophytone mantagophytes*, *Candida albicans*) and bacteria (*E. coli*, *Bacillus subtilis*, *Streptococcus faecalis*).

Onaolapo et al found the minimum inhibitory concentration between 1.56 mg/ ml to 12.5 mg/ ml of the water – methanol – chloroform- and diethyl ether/ ethanol-extract of *C. torra* L. against *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, using both cup plate and disc diffusion method.

Mukherjee et al (28) found antifungal activity of the dealcoholized extract of the leaves of *Cassia tora* Linn.

References

1. Nadkarni, K.M., Indian Materia Medica, vol I, 1993, Popular Prakashan, Bombay, pp 291.
2. Kirtikar, K.R. and Basu, B.D. , Indian Medicinal Plants, vol. II, 1988, International Book Distributors, Dehradun, pp878-9.
3. Anonymous, The Wealth of India, Raw Materials, vol II, 1950, PID, New Delhi, pp 98.
4. Naryana, C.S. and Rangaswami, S., Curr. Sci.. 1956; 25 (11):359.
5. Ghosal, S., Banerjee, P.K. and Banerjee, S.K.. Phytochemistry, 1970; 9(2):429-433.
6. Tiwari, R.D. and Behari, J.R., Plant. Med., 1972; 21(4): 393-96.
7. Acharya, T.K. and Chatterjee, I.B., Sci. Cult., 1974;40(7): 316.
8. Raghunathan, K. et al, Ind. J. Chem.,1974; 12(22): 1251-53.
9. Niranjan, G.S. and Katiyar, S.K., J. Ind. Chem. Soc., 1981; 58(1):70-72.
10. SinghR.P., *et al*, J. Inst. Chem., 1981; 55 (Pt. V): 230- 233.
11. Katoch, B.S. and Bhowmik, K.B.D., Ind. Vet. J., 1983; 60(5): 390-395.
12. Chakrabarty, K. and Chawla, H.M., Ind. J. Chem., 1983;22B(11): 1165-66.
13. Chakrabarty, K. and Chawla, H.M., Ind. J. Pharm. Sci., 1983; 45(6): 251-53.
14. Upadhyaya, S.K. *et al*., Proc. Indian Acad. Sci. (Plant Sci.), 1986; 96(4); 321-326.
15. Miralles, J., *et al*, J. Am. Oil Chem. Soc.,1989; 66(9): 1321-1322.
16. Wong, S.M., Wong, M.M., Seligmann, O. and Wagner, H., Phytochemistry, 1989;28(1): 211-214.
17. Wong, S.M., *et al*., Planta Medica, 1989; 55(3): 276-280.
18. Choi, J. S. et al, Phytochemistry, 1995; 40(3): 997-999.
19. Lee, H.T. et al, Arch. Pharmacol. Res., 1997; 20(5): 513-515.
20. Pal, M. and Pal, P.R., Indian Drugs, 1978;15(4): 49-50.
21. Maity, T.K., et al, Nat. Prod. Sci., 1997; 3(2): 122-126.
22. Maity, T.K., et al, Nat. Prod. Sci., 1998; 4(4): 226-229.
23. Maity, T.K., et al, Phytother. Res., 1998; 12(3): 221-223.
24. Sinha, M., Tiwari, P.V., South East Asian Seminar on Herbs and Herbal Medicines, Patna, Jan, 1999, pp 16-19, 55-62.
25. Singh, R.H., Khosa, R.L. and Upadhaya, B.B., J. Res. Ind. Med., 1974; 9(2): 66-67.
26. Saxena, A.P. and Vyas, K.M., J. Econ. Tax.. Bot., 1986; (2): 291-99.
27. Onaolapo, J.A. et al, Medicinal Plants: New Vistas of Research, 1993 ;11(Pt. II): 533-536.
28. Mukherjee, P.K. et al, Phytother. Res., 1996; 10(6): 520-522.